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Original Article

Predictors of mortality in children with systemic lupus erythematosus

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Abstract

Background Systemic lupus erythematosus (SLE) is a multisystem chronic disease with a relatively high mortality rate in children, despite improvements in prognosis and survival rate over the past decade. Studies on the predictors of mortality in children with SLE, especially in low- and middle-income countries, are limited.

Objective To determine the predictors of mortality of children with SLE.

Methods This was case-control study using data from medical records of children with SLE at Dr. Sardjito Hospital, Yogyakarta, Indonesia, between 2009 and 2017. Subjects were children aged <18 years diagnosed with SLE. Cases were those who died within one year of diagnosis; the controls were those who were discharged alive. From subjects' medical records, we collected clinical data including age, sex, date of diagnosis, nutritional status, anti-dsDNA antibody, antinuclear antibody (ANA), hypertension, disease activity based on the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score, proteinuria, thrombocytopenia, mortality/survival outcome, date of death, cause of death, and clinical data including fever, seizures, antibiotic used, microbial culture outcomes, and infection-related diagnoses. We performed bivariate analysis of the association between predictor variables (SLEDAI score, proteinuria, infection, hypertension, and seizures) and mortality outcome (survival or death), followed by logistic regression analysis.

Results Eighty-four patients with SLE were included, of which 72 were female. Median age at diagnosis was 14 (range 4-18) years. Twenty-three patients (27%) died within one year after diagnosis. The most common causes of death were infection and renal failure in 8/23 and 7/23 subjects, respectively. On bivariate analysis, the variables significantly associated with mortality were hypertension (OR 3.34, 95%CI 1.22 to 9.14) and infection (OR 3.71; 95%CI 1.36 to 10.12). Seizures, proteinuria, and SLEDAI score were not found to be significantly associated with mortality. On logistic regression analysis, infection was the only significant predictor of mortality (OR 3.22; 95%CI 1.15 to 9.05).

Conclusion Among the factors studied, infection is significantly associated with mortality in children with SLE. **[Paediatr Indones. 2019;59:1-6; doi: http://dx.doi.org/10.14238/pi59.1.2019.1-6].**

Keywords: *predictor; mortality; children; systemic lupus erythematosus*

Systemic lupus erythematosus (SLE) is a chronic disease of varying degrees of severity, ranging from mild to life-threatening. Over the past few years, SLE in children has become considered a fatal disease. Some studies have reported that the prognosis of SLE in children is poorer than in adults.[1] Childhood SLE is more common in females, with a female-to-male ratio of 3:1. After puberty, this ratio increases to 9:1. The incidence of

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childhood SLE is approximately 0.3 to 0.9 per 100,000 children per year, with an average prevalence of 3.3-8.8 per 100,000 children.[2] At Dr. Sardjito Hospital, Yogyakarta, a regional referral hospital, from 2015 to April 2017 new cases accounted for 10.6% of all SLE cases.[3]

The life expectancy of SLE patients has improved in recent decades. Prior to 1955, the 5-year survival rate for SLE was less than 50%. Today, the 10-year survival rate has risen to about 90%. The increase in life expectancy is due to earlier diagnosis, more aggressive treatment with agents such as corticosteroids and cytostatics, better access to hemodialysis for patients with renal failure, and better treatment of complications such as infection, hypertension, and hyperlipidemia.[4] Race, sex, age at diagnosis, thrombocytopenia, nephritis, central nervous system involvement, and disease progression are considered to be associated with mortality in patients with SLE. In a case-control study in Mexico, the main cause of mortality was infection. Other factors associated with mortality are nephritis, therapeutic steroid index, the *SLE Disease Activity Index* (SLEDAI) score, and severe infection.[5–8]

The predictors of mortality in children with SLE has not been extensively studied in Indonesia. A study of predictors of childhood SLE mortality conducted in our hospital in 2012 found that positive anti-dsDNA antibody is a prognostic factor for mortality.[9] In the present study, we aim to provide current data on the predictors of mortality in childhood SLE patients at Dr. Sardjito Hospital.

Methods

This was a case-control study of children aged one month to 18 years diagnosed with SLE based on the 1997 American College of Rheumatology (ARC) criteria who were seen at Dr. Sardjito Hospital from January 2009 to December 2017. Cases were subjects who died within one year of diagnosis. Death was established from the medical records of subjects who died at the hospital's pediatric inpatient care unit or from reports of subjects' death from their parents. The control group consisted of subjects who were discharged from the hospital alive at least one year after diagnosis.

Data collected from the patients' medical records included age, sex, date of diagnosis, nutritional status, anti-dsDNA antibody, antinuclear antibody (ANA), and clinical variables associated with SLE at the time of diagnosis, including hypertension (systolic and/or diastolic blood pressure above the 95th percentile for sex, age, and height), disease activity, proteinuria (urinary protein $\geq$0.5 g per day or $\geq$+3 using dipstick), thrombocytopenia (platelets $<$100,000/mm^3), mortality outcome (survival or death), date of death, and cause of death.[10,11] We also recorded other relevant data, such as fever, seizures, antibiotic used, microbial culture outcomes, and infection-related diagnoses.

Disease activity was assessed based on *Systemic Lupus Erythematosus Disease Activity Index* (SLEDAI), a scoring system comprising 24 items. SLEDAI scores were calculated at the point of diagnosis. A score of one to ten was classified as mild-moderate disease activity, while a score of $\geq$11 was classified as severe.[12] Infection was defined as a positive microbial culture or a record of suspected new infection necessitating sampling for culture, initiation of antibiotics, or change in antibiotic regimen. Nutritional status was determined based on the *World Health Organization* (WHO) *z*-score growth charts. We used weight-for-height charts for children aged $\leq$5 years, and body mass index (BMI)-for-age charts for children aged $>$5 years. Subjects were classified as well-nourished if weight-for-height or BMI-for-age was $>$-2 standard deviations (SD) but $\leq$+2 SD, moderately malnourished if weight-for-height or BMI-for-age was $<$-2 SD, severely malnourished if weight-for-height or BMI-for-age was $<$-3 SD, overweight if weight-for-height or BMI-for-age was $>$+2 SD, and obese if weight-for-height or BMI-for-age was $>$+3 SD.

Data was entered into *Epidata software* (Epidata Association, Odense, Denmark). Statistical analysis was performed using *SPSS version 24.0* (SPSS Inc., Chicago). Univariate analysis between predictor variables and mortality outcome was done using the chi-square test. The predictor variables analyzed were disease activity using SLEDAI score, proteinuria, infection, hypertension, and seizures. The main outcome was mortality (survival or death). Variables with a P value of $<$0.25 were entered into a logistic regression analysis. A P value of $<$0.05 was considered statistically significant.

The study protocol had been approved by the Ethics Committee of the Universitas Gadjah Mada Medical School.

Results

A total of 134 children aged <18 years with SLE sought treatment at Dr. Sardjito Hospital during the study period. Eighty-four children met the inclusion criteria; 23 died and 61 survived one year after the diagnosis. Subject recruitment flow can be seen in **Figure 1**.

Out of 84 subjects included in the study, 23 died within one year after diagnosis. The cause of death was infection in 8 subjects, renal failure in 7 subjects, cardiovascular disorders in 3 subjects, neuropsychiatric SLE in 1 subject, and other cause in 2 subjects. The remaining 2 subjects died at home and in another hospital, respectively, with unknown or untraceable causes of death. Sepsis (5/8), pneumonia (2/8), and diarrhea (1/8) were the main types of infection leading to death.

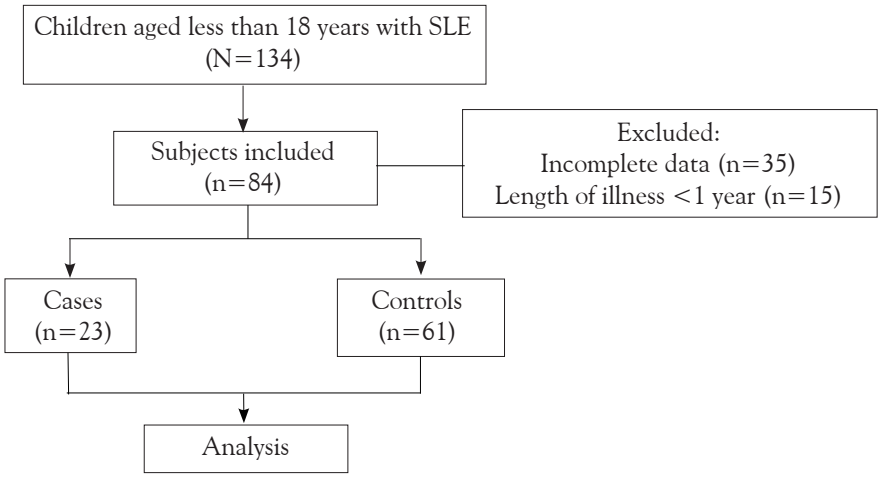

Figure 1. Subject recruitment flow

The baseline characteristics of study subjects are presented in **Table 1**. In both groups, most subjects were female. Most subjects were well-nourished; no subject was overweight or obese. Baseline characteristics appeared to be comparable between the case and control groups.

On bivariate analysis (**Table 2**), seizures (P=0.07), SLEDAI score (P=0.05), and proteinuria (P=0.09) were not significantly associated with mortality. On the other hand, hypertension (OR=3.34; 95%CI 1.22 to 9.14; P=0.02) and infection (OR 3.71; 95%CI 1.36 to 10.12; P=0.01;) significantly affected mortality. All variables studied had a P value of <0.25 and were thus included in the multivariate analysis. On logistic regression analysis, the only variable significantly associated with mortality was infection (OR 3.22; 95%CI 1.15 to 9.05; P=0.02) (**Table 2**).

Discussion

In this case-control study of factors associated with mortality in pediatric SLE, we found that hypertension and infection significantly affected of mortality. However, when all factors were considered, only infection remained as a significant associated factor. This study was done as an update to a similar study conducted at our center in 2012 by Farkhati *et al.*[9]

In the current study, we found a male-to-female ratio of 1:6. This ratio is similar to that found in Farkhati's study (1:6.9) and consistent with studies in other centers, which have reported male-to-female ratios ranging between 1:4-5 to 1:10.[6,13] The median age at diagnosis in both our case and control groups was approximately 14 (range 4 to18) years; the majority of patients in both groups were >10 years of

age (91.3% and 85.2% in the case and control groups, respectively). The median age Farkhati's study was 11.9 years.[9] A similar study in the Philippines reported a median age at diagnosis of 14 years.[14] This shows that pediatric SLE is commonly diagnosed during puberty.

Table 1. Subject characteristics

Characteristics	Died (n=23)	Survived (n=61)
Sex, n (%)		
Male	4 (17.4)	8 (13.1)
Female	19 (82.6)	53 (86.9)
Age at onset of disease, n (%)		
<10 years	2 (8.7)	9 (14.8)
≥10 years	21 (91.3)	52 (85.2)
Median age (range), years	14 (8 to 17)	14 (4 to 18)
Median time since diagnosis (range), days	62 (4-350)	1119 (365 -3772)
Median SLEDAI score (range)	19 (8-33)	13 (2-24)
Trombocytopenia		
Yes	3 (13)	13 (21.3)
No	20 (87)	48 (78.7)
ANA		
Positive	18 (78.3)	51 (83.6)
Negative	5 (21.7)	10 (16.4)
Anti ds-DNA		
Positive	17 (73.9)	47 (77)
Negative	6 (26.1)	14 (23)
Nutritional status		
Well-nourished	17 (74)	45 (74)
Moderate malnutrition	2 (9)	12 (20)
Severe malnutrition	4 (17)	4 (7)

Approximately a quarter of children with SLE died less than one year after diagnosis. The causes of death in SLE in Asia-Pacific are infections (30-80%), active SLE (19-95%), cardiovascular disorders (6-40%), and renal involvement (7-36%).[4,13,15,16] In our center, infection was the main cause of mortality in 20129 and remains the leading cause of death in the present study, accounting for 8/23 deaths, followed by renal failure (7/23) and cardiovascular disorders (3/23). The most common type of infection in this study was sepsis, which is similar to a previous report in Brazil.[6]

Although the SLEDAI score in the case group in our study was higher when compared to the control group (19 *vs.* 13), it was not significant for mortality, as well as for proteinuria. This is similar to some previous study in Malaysia.[16] Feng *et al.* in their study also reported that proteinuria and SLEDAI score were not a predictor of mortality, on the contrary it was said that seizure, as one of the manifestation of neuropsychiatry, was an independent predictor.[17] This is slightly different from our study, where the seizure are not a predictor of mortality.

Hypertension in SLE patients is associated with end-stage renal disease (ERDS) that eventually leads to death.[18,19] In the present study, hypertension was found to increase the risk of death (OR 3.34; 95%CI 1.22 to 9.14). These results are similar to those of a study in China, which reported that hypertension increased mortality risk up to threefold.[19] However, in

Table 2. Univariate and multivariate analysis of predictors and mortality among children with SLE

Parameters	Died n(%)	Survived n(%)	Bivariate analysis		Multivariate analysis	
			OR (95%CI)	P value	OR (95%CI)	P value
Hypertension						
Yes	12 (52.2)	15 (24.6)	3.34	0.02	2.83	0.05
No	11 (47.8)	46 (75.4)	(1.22 to 9.14)		(0.99 to 8.04)	
Seizures						
Yes	7 (30.4)	8 (13.1)	2.89	0.07	2.15	0.24
No	16 (69.6)	53 (86.9)	(0.91 to 9.23)		(0.59 to 7.76)	
Proteinuria						
≥+3	13 (56.5)	22 (36.1)	2.30	0.09	1.16	0.80
<+3	10 (43.5)	39 (63.9)	(0.86 to 6.17)		(0.35 to 3.92)	
SLEDAI Score						
Mild-moderate activity	22 (95.7)	45 (73.8)	7.82	0.05	5.18	0.13
Severe activity	1 (4.3)	16 (26.2)	(0.97 to 62.84)		(0.61 to 44.24)	
Infection						
Yes	14 (60.9)	18 (29.5)	3.71	0.01	3.22	0.02
No	9 (39.1)	43 (70.5)	(1.36 to 10.12)		(1.15 to 9.05)	

a previous Indonesian study, hypertension significantly increases the risk of death in lupus nephritis only when it reaches hypertensive crisis levels.[20] In our study, the association between hypertension and mortality was no longer significant upon multivariate analysis. The mechanism of hypertension in SLE is likely multifactorial and includes, among others, genetics, sex, ethnicity, the renin-angiotensin system, inflammatory cytokines, and drugs. Inflammatory cytokines play a central role in the mechanism of hypertension. Increased inflammatory cytokines will increase renal vascular resistance and reduce glomerular filtration rate (GFR). Inflammatory cytokines also mediate renal vascular changes as a result of the active renin-angiotensin system and the endothelial system, through increased oxidative stress.[21,22]

The only independent variable that remained a significant predictor of mortality after multivariate analysis was infection. Infections that occur in SLE patients may result from SLE itself or as a complication of treatment with corticosteroids and immunosuppressants. The immunocompromised condition of SLE patients brings about increased susceptibility to opportunistic infections, such as yeast infections and tuberculosis. The diagnosis of infection in SLE patients is further complicated by masking of the symptoms of infection by the clinical manifestations of SLE itself. The prevention of infection is of utmost importance for SLE patients, especially in children receiving immunosuppressant and corticosteroid treatment. Similarly, prompt and accurate diagnosis is needed, as well as adequate treatment for every SLE patient who gets an infections. Delay in the diagnosis of infections is associated with higher mortality rates in children with SLE. The most common organs affected by infection in SLE patients are the lungs, urinary tract, and joints.[6,23]

The main limitation of our study was the retrospective retrieval of information from medical records, which may lead to information bias. Since this study was based in an academic referral hospital, most cases were patients referred in poor clinical conditions. However, our results shed light on current factors affecting the mortality of children with SLE and provides data for the development of future studies on childhood SLE.

We conclude that infection is significantly associated with mortality in children with SLE. Other studied factors, including hypertension, seizures, proteinuria, and SLEDAI score are not significantly associated with mortality when all potential risk factors are taken into account.

Conflict of interest

None declared.

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Original Article

Age at menarche and early menarche among healthy adolescents

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Abstract

Background Menarche is an important indicator of female adolescents' health and also population health. Age at menarche tends to decrease in many countries. Early menarche that defined from the lowest quartile of age at menarche, associated with some physical and psychological problems. Objective To determine the mean age at menarche, the prevalence of early menarche among healthy adolescents in Surakarta and its association to nutritional status.

Methods This cross-sectional study was conducted in 5 schools (3 junior and 2 senior high schools) in Surakarta, Central Java, Indonesia, from September 2016 to March 2018, by consecutive sampling technique. We included menarched healthy female students whose parent provided informed consent and without consuming any routine medication. Data were derived from self-reported questionnaire and measurements of body weight; body height; and body mass index (BMI) by calculated based on weight/height^2(kg/m^2).

Results Of 835 eligible subjects, the mean age at menarche was 12.0 (SD 1.1) years (range 8.8-15.1 years) and the prevalence of early menarche was 11.1%. The peak of age at menarche were at 11,12, and 13 years (24.3%, 36.2%, and 23.9%, respectively) and almost 99.04% of subjects had menarche at 14 years old. The proportion of early menarche between subjects birth 1997-2001 and 2002-2007 were 8.4% and 16.1%. Early menarche associated with overweight-obese in adolescents (odd ratio 2.14; 95%CI 1.21 to 3.76).

Conclusion The mean age at menarche of healthy adolescents in Surakarta is younger than other previous studies in Indonesia. Early menarche was significantly a risk for overweight/obese in adolescents. **[Paediatr Indones. 2019;59:33-7; doi: http://dx.doi.org/10.14238/pi59.1.2019.33-7].**

Keywords: *age at menarche; early menarche; adolescent; overweight; obese*

Menstruation, especially menstrual cycle is an additional vital sign for female adolescent health.[1] Menarche, as the first menstruation, is the last pubertal sequence in adolescent girl, after telarche, adrenarche, and growth spurt.[2] Secular trends showed an earlier age at menarche in many countries, including in Indonesia females.[3-5] On the other hand, early menarche associated with the risk of a sexual health (early sexual initiation), a reproductive health (functional ovarian reserve, eclampsia), and metabolic syndrome (obesity, cardiovascular disease). Some risk behaviour and psychological problems are also associated with early menarche. [6-10]

This study aimed to determine the mean age at menarche, the prevalence of early menarche among healthy adolescents in Surakarta and its association to nutritional status.

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Methods

This cross-sectional study was conducted in 5 schools (3 junior and 2 senior high schools) in Surakarta, Central Java, from September 2016 to March 2018, by consecutive sampling technique. We included healthy female students whose parent provided informed consent and experienced at least three menstrual cycles before the study. We excluded females taking hormonal drugs, any routine medication, or had chronic diseases.

Data of age at menarche were derived from self-reported questionnaire to the nearest of month. We defined early menarche at the lowest quartile of menarcheal age (<25th percentile). Gynecologic year is an interval time between chronological age and age at menarche, and catagorised by less or more than 3 years. Measurements of body weight (BW) and height (BH) were performed 3 times and a mean value was calculated for each subject. We measured BH using stadiometer to the nearest 0.1 cm, and BW by Seca weighing scale and calculated to the nearest 0.1 kg. Body mass index was measured by pattern BW(kg)/BH^2(m^2). Body mass index was plotted to *CDC* growth chart based on sex and age (in percentile). Nutritional status was catagorised based on body mass index-for-age [CDC criteria: underweight (<5th percentile), normoweight (5th-85th percentile), overweight (85th-<95th percentile) and obese (≥95th percentile)].[11]

The data was processed using *SPSS 20.0*. Numerical data (age at menarche, weight, height, body mass index) was defined as a median. Categorical data (nutritional status, early menarche, gynecologic year) was defined as proportion/percentage. Age at menarche was described in percentage and cumulative percentage. A Chi-square test was used for analyzing the association between early menarche and nutritional status (overweight-obese vs. under-normoweight). This study was approved by Ethics Committee of the Universitas Sebelas Maret Medical School.

Results

There were 835 eligible subjects born between 1997-2007. The mean of age and BMI were 15.1 (SD 1.8) years and 20.0 (SD 3.9) kg/m^2, respectively. Birth year of subjects were from 1997-2007. The proportion of gynecologic age year less and more than 3 years were 48.5% and 51.5%. The prevalence of overweight and obese subjects were 9.3% and 1.8% (**Table 1**).

Table 1. Characteristics of subjects

Characteristics	(N=835)
Age, years	
Mean (SD)	15.1 (1.8)
Median (range)	15.1 (11.5-18.5)
Birth year, n (%)	
1997	7 (0.8)
1998	161 (19.3)
1999	136 (16.3)
2000	111 (13.3)
2001	122 (14.6)
2002	105 (12.6)
2003	94 (11.3)
2004	22 (2.6)
2005	32 (3.8)
2006	44 (5.3)
2007	1 (0.1)
Weight, kg	
Mean (SD)	49.9 (10.3)
Median (range)	48.0 (29.0-120.5)
Height, cm	
Mean (SD)	155.0 (6.4)
Median (range)	155.0 (132.0-184.4)
BMI, kg/m^2	
Mean (SD)	20.7 (3.9)
Median (range)	20.0 (11.3-49.5)
BMI percentile	
Mean (SD)	57.0 (29.3)
Median (range)	57.0 (0.0-99.6)
Nutritional status, n (%)	
Underweight (<5th)	40 (4.8)
Normoweight (5-<85th)	702 (84.1)
Overweight (85-<95th)	78 (9.3)
Obese (≥95th)	15 (1.8)
Gynecologic age, years	
≤3 years, n (%)	405 (48.5)
>3 years, n (%)	430 (51.5)

The mean age at menarche was 12.0 (SD 1.1) years with the 25th, 50th, and 75th percentiles of age at menarche were 11.0, 12.0, 12.7 years, respectively. The prevalence of early menarche was 11.1%. (**Table 2**).

Most subjects had menarche at the ages of 11 (24.3%), 12 (36.2%), and 13 (23.9%) years (**Figure 1**). We cumulatively found 99.04% of female had their menarche by the age of 14 years (**Figure 2**). The prevalence of early menarche between subjects birth 1997-2001 and 2002-2007 were 8.4% and

16.1%, respectively (**Table 3**). Early menarche was significantly associated with higher rates of overweight-obesity (P=0.008) (**Table 4**).

Table 2. Age at menarche, number of early menarche and gynecologic year among subjects

Age at menarche, years	
Mean (SD)	12.0 (1.1)
Median (range)	12.0 (8.8-15.1)
25th	11.0
50th	12.0
75th	12.7
Early menarche,* n (%)	93 (11.1)

*Early menarche: <25th percentile (<11 years of age)

Discussion

Timing of puberty has been used as an indicator of population health status. The interval time between breast budding (as the first sign of puberty in female) and menarche is approximately 2-2.5 years. Although menarche is the last stage of puberty, it is the easiest parameter to evaluate the timing of puberty.[2] Present study showed the mean age at menarche in adolescents living in Surakarta was 12.0 (SD 1.1) years. It was younger than results of Batubara study (which held in 1992-1995, 12.96 years), Sohn study (in Indonesian female born before 1990, 13.18 years) and Wahab's meta-analysis study (before 2010, 13.63 years).[3-5] Our study showed that most of Surakarta's female had their menarche at 11-13 years old. At 14 years of age,

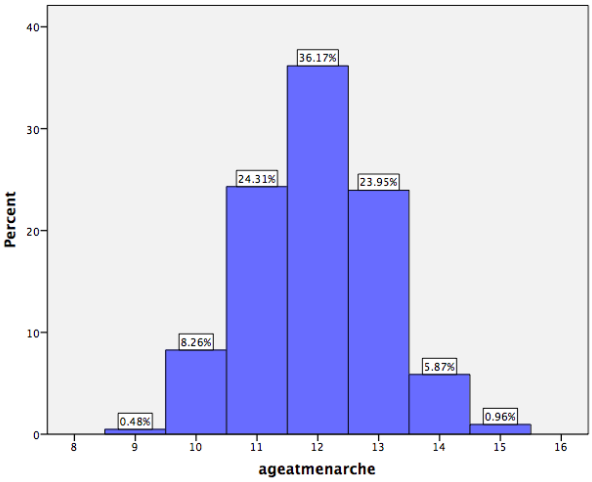

Figure 1. Ages at menarche of Surakarta girls

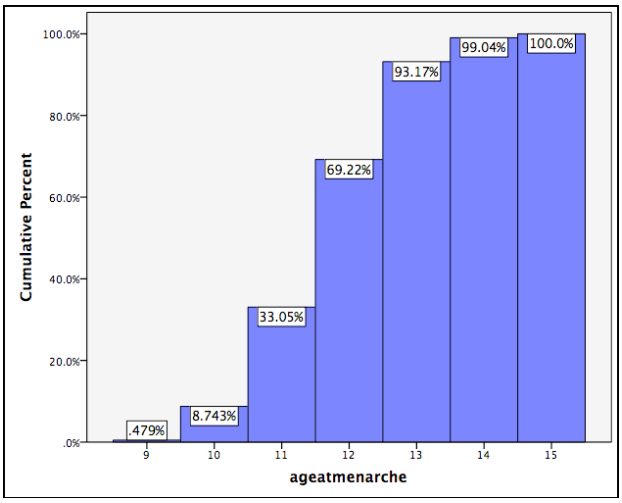

Figure 2. Cumulative percentage of ages at menarche of Surakarta adolescents

Table 3. Association between birth year and early menarche

Birth year	Early menarche	Normal menarche	Odds ratio (95% CI)	P value
2002-2007, n(%)	48 (16.1)	250 (83.9)	2.10 (1.36 to 3.24)	
1997-2001, n(%)	45 (8.4)	492 (91.6)		0.0008

Table 4. Associations between early menarche and nutritional status

	Overweight-obese (n=93)	Normo-underweight (n=742)	Odds ratio (95% CI)	P value
Age at menarche, n(%)				
Early	18 (19.4)	75 (10.1)	2.14 (1.21 to 3.76)	0.0088
Normal	75 (80.6)	667 (89.9)		

almost 99% of female have had their menarche. It is one year younger than the previous study.[4] There may some considerations with the results: first, Surakarta is a city near to Yogyakarta where the lowest mean age at menarche according to Batubara's study;[4] second, our subjects were from urban. Some studies revealed the difference of menarcheal age between urban and rural area.[12-17] Further study is needed to compare urban and rural in Surakarta area.

In our study, the proportion of early menarche from 1997-2001 to 2002-2007 was increasing (from 0.08 to 0.16). The overall proportion of early menarche in Indonesia from Wahab's study was 0.07 (95%CI, 0.05 to 0.10). The lowest one was from the Purworejo District, Central Java, (0.02; 95%CI, 0.017 to 0.022) and the highest ones were from Yogyakarta (0.12, 95%CI, 0.09 to 0.16) and Jatinangor West Java (0.14, 95%CI, 0.10 to 0.19). The proportion of early menarche in other studies (Korea, French, Saudi) were varied. The early menarche cut off was different among studies, depended on the lowest quartile of age at menarche.[5,10,16]

Several problems had been known associated with early menarche.[6-10] This study proved that early menarche twicely increased the risk of overweight and obese adolescent. Meta-analysis by Prentice concluded that early menarche increased risk of adult's obesity twice.[17] Previous studies revealed that body mass index was a risk for early menarche and vice versa early menarche associated with adult's body mass index.1[7-19] Our study could not differentiate whether the obesity occurred before or after menarche because we did not have nutritional status data before menarche. The history of small gestational age data not provided in this study could be a confounding factor related to the obesity in adolescents.

There are other limitations of our study. First, a recall bias might occured from self-reported questionnaire. Almost all subjects cannot exactly determine the time of menarcheal age. Second, the study design was cross sectional study. A cohort prospective study is needed to determine the risk of obesity in early menarche subjects with controlling the confounding factors, such as nutrition, physical activity, genetic or nutritional status before puberty. We proposed to give some attentions to early menarche in the context of obesity and other physical, and also psychological problems.

We conclude that the mean age at menarche in our study is earlier with peak at 11-13 years of age. The proportion of early menarche increases and associates with obesity in female adolescents.

Conflict of Interest

None declared.

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Original Article

Clinical spectrum and outcomes of pediatric diphtheria

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Abstract

Background Although vaccination programs have succeeded in reducing the incidence of diphtheria, it remains a health problem in Asia, including Indonesia.

Objective To investigate the clinical spectrum and outcomes of pediatric diphtheria in Wahidin Sudirohusodo Hospital.

Methods This study was a retrospective review of childhood diphtheria medical records from January 2011 to December 2017 in Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi. Recorded data consisted of age, gender, nutritional and immunization statuses, signs and symptoms, throat swab culture results, complications, and outcomes.

Results Of 28 subjects aged 9 months to 17.10 years, the majority were >5 years (57.1%) and male (60.7%). Subjects' mean age was 6.15 years and 82.1% of cases were well nourished. Overall, 85.7% had received complete immunizations, while 14.3% were not immunized, having received neither basic nor booster vaccines. The presenting manifestations were fever, pseudomembranes, and sore throat in all subjects, enlarged tonsils (78.57%), dysphagia (67.86%), cough (57.14%), headache (57.14%), hoarseness (67.86%), bull neck (25%), and myocarditis (14.3%). Most subjects had hospital stays of >10 days (67.9%). Mortality was 14.3%, usually in those admitted with a late, deteriorating condition and dying before getting optimal treatment. Poor outcome was significantly associated with the lack of basic or booster immunizations, poor nourishment, bull neck, myocarditis, and hospital stays < 5 days (P<0.05 for all).

Conclusion The clinical spectrum and outcomes of pediatric diphtheria in this study are relatively similar to reports from other hospitals. Mortality was mostly in patients who lack basic or booster immunizations, are poorly nourished, or have bull neck, myocarditis, or hospital stays < 5 days. **[Paediatr Indones. 2019;59:38-43; doi: http://dx.doi.org/10.14238/pi59.1.2019.38-43].**

Keywords: *clinical spectrum; outcome; diphtheria*

Diphtheria, taken from the Greek, "diphtera" which means leather hide, was first identified by Hippocrates.[1] It is an acute, fatal, bacterial toxin-induced disease caused by Corynebacterium diphtheria. The disease has been almost completely eradicated in developed countries, including many European nations. However, diphtheria remains an important cause of child mortality in developing countries and although the incidence has declined, still accounts for 80-90% of the global burden.[2] In 2015, India contributed 2,365 (52.21%) of the 4,530 diphtheria cases reported globally.[3] Although vaccination programs have succeeded in reducing the incidence of diphtheria worldwide, diphtheria remains a health problem, especially in Asia. The *World Health Organization* (WHO) reported that the number of diphtheria cases in 2013 was 4,680, which were widespread and mostly concentrated in the Asian continent, including India (3,313 cases), Indonesia (775 cases), Iran (190 cases),

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Pakistan (183 cases), and Nepal (103 cases). Indonesia had the second highest number of diphtheria cases.[4,5] This disease mostly occurs in children under 5 years of age, but may also occur in children over 5 years (5-19 years) and in adults.[6] Several studies have shown that low vaccination coverage, crowding, migration, or a combination of host, agent, and environmental factors, can influence the incidence of diphtheria,[7,8] in addition to nutritional status, parental behavior, and personal hygiene of children.[9]

Many outbreaks of diphtheria have been reported in various states of India in recent times.[10-14] A diphtheria outbreak of 593 cases in Indonesia was documented by several provincial health offices between 1 January and 1 November 2017, many occurring in East Kalimantan in children aged 1-10 years.[15]

Since there has been a lack of studies on childhood diphtheria in Wahidin Sudirohusodo Hospital, Makassar, we aimed to investigate the clinical spectrum and outcomes of childhood diphtheria in our facility.

Methods

This retrospective study included patients with diphtheria aged less than 18 years and hospitalized in the Pediatrics Ward of Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, from January 2013 to September 2018. Patients with incomplete medical records were excluded. All patients with suspected, probable, or confirmed cases of diphtheria based on the *WHO* definition guidelines were enrolled in the study.[16,17]

The DTP vaccination is recommended to be administered at age of 2, 3, and 4 months and booster at age of 7, 10, and 12 years, and repeated every 10 years. If a subject received basic and booster vaccination then her/his immunization status was categorized as complete. If a subject did not received either basic nor booster vaccination then her/his status was no immunization.[17]

Signs and symptoms of diphtheria included malaise, sore throat, low-grade fever, nasal discharge, bloody nasal discharge, hoarseness, cough, pain with swallowing, noisy breathing (inspiratory stridor), shortness of breath, chills, fatigue, cyanosis, headache, rapid breathing, lymphadenopathy, cardiac arrhythmia, and myocarditis.[18,19] Suggestive signs and symptoms of diphtheria, such as fever, cough, sore throat, dysphagia, difficulty swallowing and/or breathing, hoarseness, enlarged tonsils with evidence of pseudomembranes around the tonsils and surrounding areas, and bull neck were collected from subjects' medical records and further analyzed.

Pseudomembranes were defined as typical tough, gray-white membranes overlying the inflamed, edematous mucosa of the tonsils, pharynx, larynx, or trachea, strongly adherent to the underlying tissue, with attempts to dislodge it usually resulting in bleeding. Bull neck was defined as an obviously swollen neck due to swollen cervical lymph nodes, soft tissue edema and mucosal edema. Myocarditis was defined as signs and symptoms of cardiac involvement such as weak and irregular pulse, tachypnea, tachycardia, cyanosis, dyspnea, weakness, diminished heart sounds, cardiac dilation, gallop rhythm, or changes to the electrocardiograph (ECG) pattern, particularly ST-T wave changes and heart block after 1 to 2 weeks of illness.[18,20,21]

Nutritional status was based on the Waterlow (body weight/body height percentile) and *WHO* (body weight/body height SD) criteria and categorized as well-nourished [>90 / (+2 SD) - (-2 SD)], undernourished [70-90/ (< - 2 SD) - (- 3 SD)], poorly nourished [< 70/ (- 3 SD)], overweight [>110/ (>(+ 2 SD) – (+ 3 SD)], and obese [>120 / (+ 3SD)].[22,23]

Other data obtained from subjects' medical records were age, gender, nutritional status, immunization status, throat swab culture results, complications, medical interventions, and patient outcomes (survived or died). All patients were treated with appropriate antibiotics and anti-diphtheria serum (ADS) in the pediatrics ward and those with complications were treated with appropriate management in the pediatric intensive care unit based on *WHO* guidelines.[18] Data were analyzed using the *Statistical Package for the Social Sciences (Windows Version 21.0*; SPSS Inc., Chicago, IL, USA). Clinical spectrum of the childhood diphtheria patients was analyzed by means of descriptive statistics and shown as ranges, means, and percentages, whereas outcomes were analyzed using Chi-square test. Results were considered significant for P values <0.05. This study

was approved by the Ethics Committee of Wahidin Sudirohusodo Hospital, Makassar.

Results

There were 28 pediatric diphtheria patients with complete medical records during the study period, ranging in age from 9 months to 17.10 years, with the mean age of 6.15 years. The majority of patients (16/28) were above 5 years. Subjects' male: female ratio was 1.54:1, with 17/28 boys. Most patients had well-nourished nutritional status (23/28). Three patients were hospitalized in 2013, three in 2014, two in 2015, six in 2016, eight in 2017, and six in 2018.

Of 28 subjects, 24 had received complete immunizations and 4 had not been immunized. All patients presented with fever, pseudomembranes, and sore throat (28/28), followed by enlarged tonsils (22/28), dysphagia (19/28), cough (16/28), headache (16/28), hoarseness (19/28), and bull neck (7/28). Myocarditis was seen in 4/24 patients and was the only complication observed in our study. Length of hospital stay was mostly ≥ 10 days (19/28) and varied from 3 to 23 days, with a mean of 12.43 days. Mortality was 4/28 and usually in those entering the hospital with a late, deteriorating condition and dying before getting optimal treatment (**Table 1**). Poor outcome was significantly associated with a lack of basic or booster immunizations (P=0.000), poor nourishment (P=0.000), bull neck (P=0.000), myocarditis (P=0.000), and length of hospital stay < 5 days (P=0.001) (**Table 2**).

Table 1. Clinical spectrum of patients with diphtheria

Characteristics	(N=28)
Age, years	
Mean age (SD)	6.15 (3.8)
Median (range)	12 (0.9 -17.1)
Age group, n	
<5 years	12
5-10 years	13
>10 years	3
Sex, n	
Boys	17
Girls	11
Nutritional status, n	
Well-nourished	23
Undernourished	2
Poorly nourished	3
Length of hospital stay, n	
< 5 days	2
5-10 days	7
≥10 days	19
Signs and symptoms, n	
Fever	28
Sore throat	28
Dysphagia	19
Hoarseness	19
Cough	16
Headache	16
Enlarged tonsils	22
Pseudomembranes	28
Bull neck, n	
Yes	7
No	21
Myocarditis, n	
Yes	4
No	24
Vaccination, n	
Yes	24
No	4
Outcomes, n	
Survived	24
Died	4

Discussion

During the 6-year study period, 28 patients with diphtheria were admitted to Wahidin Sudirohusodo Hospital, Makassar, with an age range of 9 months to 17.10 years and mean age of 6.15 years. The majority of patients were above 5 years of age (16/28), similar to that reported by Meshram *et al.* (55.32%; mean age of 6.46 (SD 3.08) years),[24] Kole *et al.* (64.28%),[25] Basavaraja *et al.* (74.1%),[26] and Bandichhode *et al.* (66.66%).[27] Another study documented that their youngest patient was also 9 months of age and the others mostly between 5 to 10 years of age.[28]

There were more boys (17/28) than girls (11/28) in our study, with a boy to girl ratio of 1.54:1, which was slightly different from previous studies with nearly equal boy to girl ratio (0.95:1).[24,29] However, Khan *et al*. noted more boys (69.64%) than girls (30.36%), with a ratio of 2.29:1.[28]

In our study of 28 patients, 24/28 had received complete immunizations, while 4/28 had received neither basic and nor booster immunizations. Different findings were reported by Meshram *et al.* (4.25% fully immunized, 57.45% partially immunized, and

Table 2. Outcomes of patients with diphteria

Clinical variables	Outcomes			P value
	Survived (n=24)	Died (n=4)	Total (N=28)	
Age, n				0.156
<5 years	12	0	12	
5-10 years	10	3	13	
>10 years	2	1	3	
Sex, n				0.636
Male	15	2	17	
Female	9	2	11	
Nutritional status, n				0.000
Well-nourished	22	1	23	
Undernourished	2	0	2	
Poorly nourished	0	3	3	
Length of hospital stay, n				0.001
<5 days	0	2	2	
5-10 days	6	1	7	
≥10 days	18	1	19	
Bull neck, n				0.000
Yes	3	4	7	
No	21	0	21	
Myocarditis, n				0.000
Yes	0	4	4	
No	24	0	24	
Complete vaccination, n				0.000
Yes	24	0	24	
No	0	4	4	

38.30% unimmunized),[24] Basavaraja *et al.* (48.3% fully immunized and 48.3% unimmunized),[26] and Ratageri *et al.* (42% fully immunized, 16% partially immunized, and 42% unimmunized).[30] Four patients in this study were unimmunized maybe due to missing the immunization schedule in Makassar as well as some districts in South Sulawesi, possibly because of lack of awareness, avoiding immunizations, migration, decreased enthusiasm to receive periodic routine immunizations, lack of emphasis on booster vaccination at school entry, not opening a multi-dose vial if not enough children are present, or postponing vaccination in children with minor illnesses.[31,32]

All patients in our study presented with fever, pseudomembranes in the throat, and sore throat, followed by enlarged tonsils, dysphagia, cough, headache, hoarseness, and bull neck. Ratageri *et al.* reported that the most common clinical presentation in their patients were pseudomembranes (100%), fever (92.8%), cervical lymphadenopathy (92.8%), followed by sore throat (64.2%), neck swelling (42.8%), dysphagia (35.7%), bull neck (28.5%), and halitosis (21.4%),[30] whereas Meshram *et al.* reported throat pain (95.74%), enlarged/congested tonsils (80.85%), respiratory difficulty (68.08%), dysphagia (59.57%), bull neck (48.94%), and voice change (36.17%).[24]

In our study, myocarditis was found in 4/28 of patients, as the only complication observed, but Meshram *et al.* found myocarditis in 42.55% of patients[24] and various studies in India found incidences of diphtheria myocarditis from 16 to 66%.[33-35] Basavaraja *et al.* showed that out of 45.16% patients with bull neck, 71.4% died; and out of 41.9% patients with myocarditis, 76.9% died.[26] However, in our study out of 25% patients with bull neck, 57.1% died, whereas all patients with myocarditis died. Khan *et al.* reported that out of 16% of their patients with myocarditis, 8.92% died.[28] The mortality from a study in India was 5%.[25] Cardiac involvement in diphtheria is caused by exotoxins produced by *Corynebacterium diphtheria*.[36,37] Mortality in our study was higher than that reported by previous studies and may be due to our different sample size or that some of our patients entered the hospital in a late, deteriorating condition

and died before getting optimal treatment.

Poor outcome of the patients in our study was significantly associated with a lack of basic or booster immunizations (P=0.000), poor nourishment (P=0.000), bull neck (P=0.000), myocarditis (P=0.000), and length of hospital stays < 5 days (P=0.001). Children with poor nutritional status may have immune deficiencies resulting in reduced response to vaccines.[38,39] Therefore, the key to preventing mortality from diphtheria in children is simultaneous improvement in their nutritional status and getting complete immunizations.

In conclusion, the clinical spectrum and outcomes of pediatric diphtheria in Wahidin Sudirohusodo Hospital, Makassar are relatively different to those reported from other countries. The mortality rate of our patients is 14.3%, most of whom had not received basic or booster immunizations, were poorly nourished, and had bull neck, myocarditis, and length of hospital stays < 5 days. The relatively high mortality is due to myocarditis, which highlights the necessity of early diagnosis and prompt treatment with ADS to reduce mortality. In addition, immunizations (basic or booster) should be encouraged in Makassar and South Sulawesi. We recommend that vaccination schedules include boosters, based on accurate surveillance, to reach high vaccination coverage in order to prevent diphtheria outbreaks and control the disease.

Conflict of Interest

None declared.

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Original Article

Predictors of early growth failure in preterm, very low birth weight infants during hospitalization

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Abstract

Background Preterm, very low birth weight (VLBW) infants experience intrauterine nutritional deficits and perinatal comorbidities that may impair early growth parameters. Early growth failure has detrimental effects on later growth and neurodevelopment in childhood.

Objective To analyze predictors of early growth failure in preterm, VLBW infants and differences in early growth parameters between small-for-gestational age (SGA) and appropriate-for-gestational age (AGA) infants.

Methods This retrospective cohort study was conducted at Dr. Sardjito Hospital, Yogyakarta from 2011 to 2016. Subjects were preterm infants, with birth weights of 1,000-1,499 g. Twins, those who died during hospitalization, were discharged against medical advice, or had incomplete medical records were excluded. Adequate intrauterine growth was determined by the Lubchenco table criteria. Growth parameters and perinatal comorbidities were collected from medical records. Growth failure was defined as discharge weight less than 10th percentile of the Fenton growth curve. Bivariate and multivariate analysis were used to analyze potential predictive factors of early growth failure.

Results Of 646 preterm, VLBW infants during the study period, 398 were excluded. Respiratory distress and SGA were predictors of early growth failure (AOR 6.94; 95%CI 2.93 to 16.42 and AOR 34.44; 95%CI 7.79 to 152.4, respectively). Mean weight velocities in SGA and AGA infants were not significantly different [16.5 (SD 5.9) and 17.5 (SD 5.3) g/kg/day, respectively; (P=0.25)]. Median time to regain, time to reach full feeding, and time to reach 120 kcal/kg/day were also not significantly different between SGA and AGA infants.

Conclusions SGA and respiratory distress are predictors of early growth failure in preterm, VLBW infants during hospitalization. The SGA infants grow slower than AGA infants. **[Paediatr Indones. 2019;59:44-50; doi: http://dx.doi.org/10.14238/pi59.1.2019.44-50].**

Keywords: *SGA-AGA; growth failure; preterm; VLBW*

As the biggest proportion of the NICU population, preterm and VLBW infants are susceptible to perinatal comorbidities, as well as growth and neurodevelopmental impairment.[1] About 4.7% of all births are VLBW infants, and Indonesia is included in the top ten nations with the highest prevalence of LBW (10.2%).[2,3] Preterm, VLBW infants may be further described as either SGA or AGA. Most SGA infants experienced intrauterine growth restriction (IUGR) and nutritional deficits.[4,5] Due to metabolic and gastrointestinal immaturity, compromised immune function, and other complicating medical conditions, nutritional deficits may continue during the early weeks after birth, impairing the growth rate. Perinatal comorbidities, such as patent ductus arteriosus (PDA), neonatal sepsis, necrotizing enterocolitis (NEC), respiratory distress, and anemia, aggravate the problem. Intrauterine chronic hypoxia in SGA infants results in maladaptation and ineffective energy utilization. Their thin, subcutaneous fat layer leads

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to higher energy reserve loss and hypothermic stress leads to higher basal metabolic rates.[5,6]

The early postnatal growth target of preterm infants is to reach a growth rate similar to intrauterine growth at the same gestational age.[7] Weight velocity, time to regain birth weight, time to reach full feeding, and time to reach 120 kcal/kg/day are some growth parameters that may be impaired in the early weeks, resulting in growth failure, defined as a discharge weight $<10^{th}$ percentile according to Fenton growth charts by chronological age.[8,9] The incidence and predictors of early postnatal growth failure among preterm, VLBW infants have not been well studied in Indonesia. Early postnatal growth failure may affect long term neurodevelopmental outcomes and cause persistent growth failure.[10,11] The aims of this study were to analyze potential predictive factors of early growth failure in the perinatal period of preterm, VLBW infants and to analyze differences in early growth parameters between SGA and AGA subjects.

Methods

A retrospective cohort study based on medical records was done in Perinatology Division of Dr. Sardjito General Hospital, Yogyakarta from 2011 until 2016. Subjects were infants with gestational age < 37 weeks, birth weight of 1,000-1,499 g weighed within 1 hour after birth for Sardjito deliveries and 24 hours for out-of-Sardjito deliveries. Gestational age was determined by calculating Dubowitz score, or Ballard score for very ill infants.[12] Twins, those who died during hospitalization, were discharged against medical advice, had gastrointestinal surgery, or syndromes were excluded from this study.

Sample size was calculated using an unpaired, categorical analysis formula, with 20% proportion considered to be significantly different between the affected and not affected groups. Adequate intrauterine growth was based on Lubchenco table criteria.[13] Subjects were subcategorized as SGA (birth weight $<10^{th}$ percentile) or AGA (birth weight between 10^{th} and 90^{th} percentiles), based on sex and gestational age. Recorded perinatal comorbidities, such as PDA, neonatal sepsis, respiratory distress, NEC, and anemia requiring transfusion were analyzed as possible predictors of postnatal growth failure. Echocardiography was done to assess for PDA. Neonatal sepsis was defined by fulfilling 4 of 7 clinical criteria (abnormal heart rate, abnormal respiration rate, lethargy, jaundice, feeding intolerance, thermoregulation instability, and abnormal laboratory finding). Respiratory distress was defined as needing mechanical ventilation in the first 24 hours, neonatal pneumonia, ventilator dependency of >72 hours, meconium aspiration syndrome, and/or oxygen dependence for more than 28 days. Necrotizing enterocolitis (NEC) was defined by the pertinent clinical symptoms and revealed by plain, abdominal X-ray. Anemia was defined as low hemoglobin level which caused hemodynamic instability and needed packed red cell (PRC) transfusion. Growth failure was defined as discharge weight based on chronological age and sex of $<10^{th}$ percentile on the Fenton curve.[8,13] Bivariate analysis with Chi-square test followed by multivariate analysis with logistic regression test were used to assess the main possible predictors of growth failure during hospitalization. Predictive factors from multivariate analysis with P values < 0.05 were used for making mathematic models to calculate probability. Relative risk was calculated by comparing each probability based on clinical characteristics in preterm, VLBW infants. This study was approved by the Ethics Committee of Universitas Gadjah Mada Medical School, Yogyakarta.

Results

There were 636 preterm, VLBW infants during the 6-year study period in the Perinatology Ward of Dr. Sardjito General Hospital, of whom 238 were eligible for analysis and 398 were excluded (301 died, 43 twins, 39 discharged against medical advice, 3 syndromes, 12 incomplete medical records). Baseline characteristics of subjects are described in **Table 1**.

The SGA subjects had median gestational age 3 weeks older than AGA infants. Both birth weight and discharge weight in SGA group were significantly lower than those of the AGA group (mean difference of birth weight was 42.2 g). Differences in growth parameters between SGA and AGA subjects are described in **Table 2**.

Incidence of growth failure was 84.5%. Only

Table 1. Baseline characteristics and growth parameters of preterm, VLBW infants

Characteristics and growth parameters	Preterm VLBW (N=238)
SGA, n (%)	120 (50.4)
Male sex, n (%)	122 (51.3)
Gestational age, n (%)	
< 32 weeks	105 (44,1)
≥ 32-36 weeks	133 (55.9)
Type of delivery, n (%)	
Vaginal	92 (38.7)
C-section	146 (61.3)
Mean birth weight (SD), g	1,271.7 (138.6)
Mean discharge weight (SD), g	1,682.5 (182.3)
Discharge weight, n (%)	
< 3rd percentile	183 (76.9)
Between 3rd and 10th percentile	18 (7.6)
≥10th percentile	37 (15.5)
Median length of stay (range), days	33 (13-95)
Neonatal asphyxia, n (%)	106 (44.5)
Perinatal comorbidities	
Neonatal sepsis, n (%)	224 (94.1)
Positive culture, n (%)	108 (48.2)
PDA, n (%)	24 (10.1)
Respiratory distress, n (%)	136 (57.1)
NEC, n (%)	18 (7,6)
Anemia, n (%)	109 (45.8
Maternal problems	
Preeclampsia/eclampsia, n (%)	109(45.8)
Hypertension, n (%)	67 (28.2)
HELLP/partial HELLP, n (%)	45 (18.9)
Diabetes melitus,n (%)	4 (1.7)
Cardiac problem, n (%)	17 (7.1)
Mean weight velocity (SD), g/kg/day	16.9 (5.3)
Median time to regain birth weight (range), days	11 (3-42)
Median time to reach full feeding (range), days	16 (5-57)
Median time to reach 120 kcal/kg/day (range), days	20 (8-53)

HELLP: hemolysis, elevated liver function, low platelet

1.7% of all SGA, preterm, VLBW subjects reached discharge weight ≥ 10th percentile, while the rest stayed in the < 3rd percentile of the Fenton growth curve according to their chronological age. Subjects without growth failure regained birth weight, reached full feeding, and reached 120 kcal/kg/day faster than subjects without growth failure. They also had significantly higher mean weight velocity than those with growth failure (**Table 3**).

Bivariate analysis followed by multivariate analysis was used to compare independent variables (SGA and perinatal comorbidities) that may contribute to postnatal growth failure during hospitalization (**Table 4**). Logistic regression revealed that SGA and respiratory distress were the significant predictors of growth failure in preterm VLBW infants. Based on probability comparison from a mathematic model/y (using constant from logistic regression for predictive study), the relative risk was calculated using the following formula:

$$y = -0.109 + (3{,}539 \times SGA) + (1{,}937 \times \text{respiratory distress})$$

$$\text{probability} = \frac{1}{1+\exp[-(y)]}$$

(SGA: Yes=score 1, No=score 0; respiratory distress: Yes=score 1, No=score 0)

The relative risk of preterm, SGA, VLBW infants with respiratory distress suffering growth failure when they were discharged was 2.1 times higher than in AGA, VLBW infants without respiratory distress.

Discussion

In this study, 301 (47.3%) infants died during hospitalization within 6 years period. Only 5% of SGA, VLBW infants with less than 32 weeks gestational age survived until discharge. The high mortality rate was caused by complex perinatal comorbidities and younger gestational age. Tsai et al. reported that the mortality rate of SGA, VLBW with less than 32 weeks gestational age was significantly increased (OR 1.89; 95%CI 1.39 to 2.58).[15]

Surviving preterm, VLBW infants who passed critical phase in early life created specific proportion. As expected, the AGA group was dominated by infants of 28 to 32 weeks gestational age, while the SGA group was predominantly infants of 34 to <37 weeks gestational age. This composition was similar to another Perinatology Unit/NICU of a tertiary hospital, with a high mortality rate (50%) and median birth weight of 1,540 g in VLBW infants >32 weeks gestational age.[13]

The most common maternal problems in our study were preeclampsia/eclampsia and hypertension. These conditions are related to placental insufficiency that cause intrauterine growth retardation, such that the SGA proportion are higher than that of AGA. In the general population, SGA is at about 10-15%, much

Table 2. Differences in early growth parameters between SGA and AGA subjects

Parameters	SGA (n=120)	AGA (n= 118)	P value
Median gestational age (range), weeks	33 (30-36)	30 (27-33)	0.01
Mean birth weight (SD), g	1,250.76 (143.22)	1,292.96 (130.93)	0.02
Male sex, n (%)	61 (50.8)	61 (52.1)	0.49
Median time to regain birth weight (range), days	11 (3-42)	12 (3-35)	0.18
Mean weight velocity (SD), g/kg/day	16.50 (5.99)	17.52 (5.32)	0.25
Median time to reach full feeding (range), days	16 (6-38)	16 (5-57)	0.49
Median time to reach 120 kcal/kg/day (range), days	20 (8-51)	21 (9-53)	0.43
Mean discharge weight (SD), g	1,642.52 (133.25)	1,723.05 (214.46)	0.01
Median length of stay (range), days	32 (13-79)	34 (13-95)	0.73

Table 3. Comparison of growth parameters in subjects with and without postnatal growth failure

Growth parameters	With growth failure (n=201)	Without growth failure (n=37)	P value
Mean time to regain birth weight (SD), days	12.6 (6.2)	10.2 (3.9)	0.04
Mean time to reach full feeding (SD), days	18.7 (8.2)	15.3 (4.7)	0.055
Mean time to reach 120 kcal/kg/day (SD), days	22.9 (9.4)	18.9 (5.9)	0.03
Mean weight velocity (SD), g/kg/day	16.5 (5.4)	18.8 (4.7)	0.02

Table 4. Predictive factors of early postnatal growth failure in preterm, VLBW infants

Predictor factors	Growth failure (n=201)				
	Bivariate analysis			Multivariate analysis	
	n (%)	OR (95% CI)	P value	adj OR (95% CI)	P value
SGA	118 (58.7)	24.88 (5.82 to 106.31)	0.001	34.44 (7.79 to 152.4)	0.001
Male sex	108 (53.7)	1.91 (0.93 to 3.92)	0.055	1.89 (0.81 to 4.42)	0.14
PDA	22 (10.9)	2.15 (0.48 to 9.56)	0.24	1.88 (0.35 to 10.03)	0.46
Neonatal sepsis	190 (94.5)	1.52 (0.40 to 5.75)	0.38		
Respiratory distress	126 (62.7)	4.54 (2.08 to 9.89)	0.001	6.94 (2.93 to 16.42)	0.001
Anemia	93 (46.3)	1.13 (0.56 to 2.29)	0.44		
NEC	16 (8.0)	1.51 (0.33 to 6.88)	0.45		
T120 > 14 days	161 (80.1)	0.78 (0.30 to 1.99)	0.39		

PDA: patent ductus arteriosus; NEC: necrotizing enterocolitis; T120: time to reach 120 kcal/kg/day

lower than our findings. Our high SGA proportion may have been because there were many referral cases to our hospital, due to maternal and perinatal complications.

The most common perinatal comorbidity in this study was neonatal sepsis (94.1%), much higher than studies by Lima *et al.*[16] (4.6%) and Shan *et al.* (56.8%).[17] Neonatal sepsis is caused by the interaction of internal factor (immature immune system) and external factors, such as inappropriate infectious

transmission/termination practices (deemed to be in part due to poor hand-washing compliance in the nursery, high room density, frequency of room fogging, and selection of definitive antibiotics.) In addition, a diagnosis of neonatal sepsis in this study required a minimum of 4 from 7 criteria. Blood culture was performed only in 73.9% of sepsis patients. About 40% of all neonatal sepsis cases had negative blood culture. These results are indicative of the difficulty of prescribing appropriate antibiotics due to the lack of a definitive cause of sepsis. Prolonged sepsis may affect infant growth rate during hospitalization.

In this study, subjects' mean weight velocity was 16.8 (SD 5.33) g/kg/day, which was similar to growth targets of NICUs worldwide.[18] Mean weight velocity in our subjects was higher than a Brazilian study [9.3 (SD 2.3) g/kg/day]. This result proved that Indonesian preterm VLBW infants had same potential growth rate as found in other developing country.[19] Mean of weight velocity in the subgroup without growth failure was 2.3 g/kg/day higher than the subgroup with growth velocity failure (P=0.015). Based on these findings, we have to evaluate weight velocity weekly from the time they regained their birth weight, so that we can recognize growth impairment earlier.

Median time to reach full feeding was 16 days for both SGA and AGA groups. A previous study reported that the mean time to reach full feeding in VLBW infants was 15 days.[20] Median time to reach 120 kcal/kg/day in SGA and AGA was 20 and 21 days, respectively, similar to Anchieta *et al*., who reported the highest growth rate in the third week of life.[21] There was no significant difference between time to regain birth weight, time to reach full feeding, or time to reach 120 kcal/kg/day between the SGA and AGA groups. This finding means that discharge weight was determined by adequate intrauterine growth and weight velocity in the early weeks of life.

The incidence of growth failure in this study (84.5%) was much higher than in studies by Lima *et al.*[16] (26%) and Marks *et al.* (10.6%).[22] This finding may have been due to the higher SGA proportion in our study than the previous ones, while growth failure was experienced mostly by SGA infants. Variations in growth failure incidence may also have been affected by study location, complexity of medical condition, gestational age, and applied nutritional management guidelines.[23] In our center, nutritional practices for SGA and AGA preterm, VLBW infants are not different.

Mean weight velocity of the SGA group was less lower than that of the AGA group (P=0.25). Ineffective energy utilization and energy deficit due to perinatal comorbidities in SGA infants are basic causes of growth impairment. In addition, basal metabolic rate in SGA is generally higher than in AGA infants. The minimal energy reserve in SGA infants is due to the thin layer of fat that increases heat loss and results in greater energy expenditure.[24] Postnatal growth failure is due to a complex interaction of comorbidities which lead to energy expenditure and endocrine function abnormalities, central nervous system impairment and immaturity, as well as immature sucking and swallowing reflexes. Ultimately, inadequate nutrition and energy deficit during the first weeks of life are considered to be most responsible for postnatal growth failure.[23]

Respiratory distress was a perinatal comorbidity that was significantly associated with postnatal growth failure during hospitalization. Infants with respiratory distress have trouble consuming liquids, including enteral feeding as their primary energy source. It also increases the basal metabolic rate, aggravating the total energy deficit in preterm, VLBW infants.[20,23] Other perinatal comorbidities, such as neonatal sepsis, PDA, anemia, and NEC were not significantly associated with growth failure in preterm, VLBW infants in our study. This finding was contrast to a previous study that reported anemia requiring transfusion related with delayed time to regain.[26] The lack of an association could have been due to an immediate hemoglobin correction to stabilize the hemodynamics at the time of diagnosis, hence, the growth parameter was just transiently impaired. In our study, the complexity and synergy of perinatal comorbidities caused a high incidence of growth failure, while single perinatal comorbidities were not significantly associated with growth failure.

The high incidence of postnatal growth failure may have also been influenced by low calorie intake during the transition period from parenteral to full enteral feeding. Based on our findings, calorie intake decreases about 10-20 kcal/day during the transition period. Energy deficits during the transition period may only maintain the status quo or even cause decreased growth rate. Inadequate energy and protein intake

during the transition period contribute to the risk of growth failure during hospitalization.[27] The time interval from regaining birth weight until reaching full feeding and nutrition for growth at 120 kcal/kg/day was longer in our subjects than in a previous study.[28] Prolonged energy and calorie deficits in preterm, VLBW in that status quo interval (while they had already been ready for increasing body weight) cause higher growth failure in our study.

Other than SGA and respiratory distress, we need to study other factors that may contribute to growth failure in preterm, VLBW infants during hospitalization, such as early enteral feeding and monitoring the application of nutritional practices. Our study had several weakness as the retrospective design might have resulted in bias in data collection, and we only analyzed body weight to evaluate growth failure. Our study was the first in Indonesia to review detailed growth parameters in preterm, VLBW infants during early life. This study also reported on the difference in early growth parameters between SGA and AGA infants.

In conclusion, SGA and respiratory distress are significant predictors of early growth failure in preterm, VLBW infants during hospitalization. SGA infants grew less slower than AGA infants. These findings imply that low weight velocity in weekly monitoring was an appropriate warning sign of postnatal growth failure. We should optimize the interval between weight regain time, time to reach full feeding, and time to reach 120 kcal/kg/day, in order to prevent prolonged nutritional deficits. Early nutritional management targets in SGA, preterm, VLBW infants aim to maintain weight gain in parallel with the growth curve and prevent severe postnatal growth failure (discharge weight $<3^{rd}$ percentile).

Conflict of Interest

No conflict of interest.

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Original Article

Correlation of serum albumin level with platelet count and aggregation in children with nephrotic syndrome

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Abstract

Background Nephrotic syndrome (NS) is the most common renal disease among children, and contributes to life-threatening complications such as thromboembolic disease. Platelets are considered to be important agents in thrombotic events among NS patients. The gold standard assessment for platelet aggregation is the use of adenosine diphosphate (ADP) agonist, however, it is expensive and not accessible in smaller health facilities. Thus, other thrombosis parameters are needed. Previous studies suggested that low albumin increased the risk of thrombosis in NS patients.

Objective To assess for a possible correlation between albumin level and platelet count as well as platelet aggregation.

Methods This cross-sectional study was conducted in children with nephrotic syndrome who were admitted to the Pediatric Nephrology Department in Dr. Hasan Sadikin General Hospital, Bandung, West Java, from November 2017 to March 2018. Subjects were selected by consecutive sampling. Serum albumin, platelet count, and platelet aggregation were measured. Statistical analysis was conducted by Spearman's test.

Results A total of 32 patients participated in the study, with mean age of 109 (SD 7.4) months. Most subjects were male (56%). Subjects' mean serum albumin level was 2.06 (SD 1.23) g/dL; mean platelet count was 453,062.5 (SD 187,443.90)/mm3; and mean platelet aggregation values for ADP agonist concentrations of 10, 5, 2.5, and 1 μM were 86.8 (SD 8.63)%, 82.4 (SD 15.33)%, 66.6 (SD 24.90)%, 34.95 (SD 31.69)%, respectively. Partial correlation analysis revealed significant negative associations between albumin and platelet count as well as platelet aggregation assessed with 1 μM of ADP concentration ($P<0.001$), with Spearman correlation coefficients of -0.641 and -0.634, respectively.

Conclusion Serum albumin level has a moderately negative correlation with platelet count and platelet aggregation value. **[Paediatr Indones. 2019;59:7-12; doi: http://dx.doi.org/10.14238/pi59.1.2019.7-12].**

Keywords: *albumin; nephrotic syndrome; platelet aggregation value;platelet count*

Nephrotic syndrome (NS) is the most common renal disease among children. A UK and US study stated the NS incidence as 2-7 new cases per 100,000 children annually, with prevalence around 12-16 cases per 100,000 children.[1] In Indonesia, new cases of NS among children below 14 years of age were about 6 per 100,000 children annually in 1992.[2] Nephrotic syndrome may contribute to life-threatening complications during its course, including thromboembolic disease, such as deep vein thrombosis with or without pulmonary emboli, infection, as well as iatrogenic complications such as immunosuppression or bone density loss due to long-term use of steroids, and acute kidney injury which may lead to chronic kidney disease.[1,3]

Thromboembolic events are rare but generally fatal for NS patients.[4,5] The risk of thrombosis in veins and arteries is caused by increased platelet count, platelet hyperaggregation, release of active substances, elevated platelet surface mediator

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expression, and loss of low molecular weight proteins. Platelet are considered as an important agent of thrombosis among NS patients.[6] The gold standard for platelet aggregation requires the use of adenosine diphosphate (ADP) agonists, which are dense granule degranulation products that directly induce platelet aggregation, unlike thrombin and collagen which only trigger ADP and TXA2 release.[7] However, this examination may be affected by various medications such as NSAIDs, platelet receptor antagonists (*Abciximab, Clopidogrel*), glycoprotein IIb/IIIa inhibitor (*Eptifibatide*), phosphodiesterase inhibitor (*Dipiridamol*), anticoagulants (heparin, warfarin), diuretics, and vasodilators.[8] The ADP agonist test also may require a larger blood specimen (450 μL of blood), can only be conducted in major laboratories, has a high predicted cost, and requires a long time to process the specimen.[9] Other modalities with lower cost, higher availability, and easier procedures are needed to predict thrombosis events.

During relapse, serum albumin level decreases due to urinary excretion. Recent studies showed that low albumin level may elevate the venous thromboembolic rate by 2 to 5.8 times.[10] Regarding the previous studies about the similar topic, this is the first study to assess for possible correlations between serum albumin level and other thrombosis parameters, such as platelet count and platelet aggregation value in pediatric NS patients.

Methods

This cross-sectional study was conducted in the Pediatric Inpatient Ward and Pediatric Outpatient Clinic, Dr. Hasan Sadikin General Hospital, Bandung, West Java, between November 2017 – March 2018. This study was approved by the Health Studies Ethics Committee at Dr. Hasan Sadikin General Hospital, Bandung.

Subjects were selected by consecutive sampling. Children diagnosed with NS and whose parents provided written informed consent were included in this study. Patients with previous thrombotic events or hepatic function disorders were excluded. Patients who underwent treatment with thrombolysis, fibrinolysis, or NSAIDs were also excluded.

Serum albumin level was measured by a *UV-VIS Spectrophotometer*, while platelet count was assessed by *Coulter* counter. Platelet aggregation was measured as the amount of ADP agonist needed to induce aggregation, using the Helena AggRAM test kit and software.

Data processing and analysis was done with *SPSS 24.0 for Windows*. Collected data were noted on the research form, edited, and coded prior to data analysis. Spearman's test was used to analyze for correlations between serum albumin level and platelet count, as well as platelet aggregation. Results with P values <0.05 were considered to be statistically significant.

Results

A total of 32 patients participated in this study, with an age range of 17 to 210 months. Demographic characteristics are shown in **Table 1**. Most subjects were male and the mean patient age was 109 months (9 years and 1 month). Most patients were diagnosed as having resistant NS (21 subjects), followed by frequently- relapsing NS (6 subjects).

Table 1. Demographic characteristics of subjects

Characteristics	(N=32)
Sex, n	
Male	18
Female	14
Age, months	
Median (range)	102.5 (17-210)
Mean (SD)	109 (57.4)
Diagnosis, n	
First episode of NS	3
Frequently-relapsing NS	6
Steroid-dependent NS	2
Steroid-resistant NS	21

Serum albumin level, platelet count, and platelet aggregation values are shown in **Table 2**. Data on serum albumin level and platelet aggregation by way of ADP concentrations of 5 μM, 2.5μM, and 1 μM were not normally distributed ($P<0.05$), while data on platelet count and platelet aggregation value of 10 μM ADP concentration were normally distributed. A typical linear pattern of platelet aggregation and ADP concentration were observed in this study.

Table 3 shows the correlation between variables

studied. We noted that age and albumin had a significant positive correlation (r=0.462; P=0.008). However, age and platelet aggregation value showed a significant negative correlation (r=-0.353; P=0.048) with ADP concentration of 1 μM. Serum albumin and platelet count had a significant negative association (r=-0.617; P<0.001), while albumin and platelet aggregation value with 1 μM ADP concentration had a significant negative association (r=-0.658; P<0.001), suggesting that lower serum albumin level was associated with increasing platelet count and platelet aggregation. Partial correlation analysis between serum albumin level and platelet count with age as confounding factor is shown in **Table 4**.

Serum albumin had negative correlations with platelet count and platelet aggregation values assessed with ADP concentration of 1 μM. According to Guilford correlation criteria, the correlation between serum albumin level and platelet aggregation value was moderate (r>0.6).

Figure 1 shows the negative correlation between serum albumin and platelet count. There was a negative central trend with R Sq linear of 0.392. Hence, lower serum albumin was correlated with higher platelet count. **Figure 2** shows the negative correlation between serum albumin and platelet aggregation. There was a negative central trend with R Sq linear of 0.357. Hence, lower serum albumin was correlated with higher platelet aggregation.

Discussion

The majority of study subjects were male (18 boys; 56%); the male: female ratio was 1.3:1. A previous study also noted that NS was more common in males compared to females.[12] The mean age of subjects' was 109 months (9 years and 1 month old), which was

Table 2. Serum albumin level, platelet count, and platelet aggregation in pediatric NS patients

Variables	Mean (SD)	Median (range)	P value
Albumin serum, g/dL	2.06 (1.23)	2.20 (0.4-4.10)	0.015*
Platelet count, /mm^3	453,062.5 (187,443.90)	410,000 (160,000-1,022,000)	0.079
Platelet aggregation assessed by ADP agonist, %			
10 µM	86.8 (8.63)	87.55 (68-99.70)	0.080
5 µM	82.4 (15.33)	85.80 (33.70-101.30)	0.002*
2.5 µM	66.6 (24.90)	70.65 (14.60-100.70)	0.002*
1 µM	34.95(31.69)	19.95 (0.70-99.60)	<0.001*

Note: * Shapiro-Wilk test

Table 3. Analysis of possible correlations between study variables

Variables	Correlation coefficient (r_s)	P value
Age and albumin	0.462	0.008
Age and platelet count	-0.253	0.163
Age and platelet aggregation		
10 µM	0.156	0.395
5 µM	0.087	0.636
2.5 µM	-0.023	0.899
1 µM	-0.353	0.048
Albumin and platelet count	-0.617	<0.001
Albumin and platelet aggregation assissed by ADP agonist		
10 µM	-0.139	0.448
5 µM	-0.196	0.283
2.5 µM	-0.281	0.120
1 µM	-0.658	<0.001

Note: r_s = Rank Spearman correlation coefficient

Table 4. Partial correlation analyses between serum albumin level and platelet count as well as platelet aggregation, with age as a confounding variable

Correlation	Correlation coefficient	P value
Albumin and platelet count	-0.641	<0.001
Albumin and platelet aggregation assessed by ADP agonist concentration of 1 µM	-0.634	<0.001

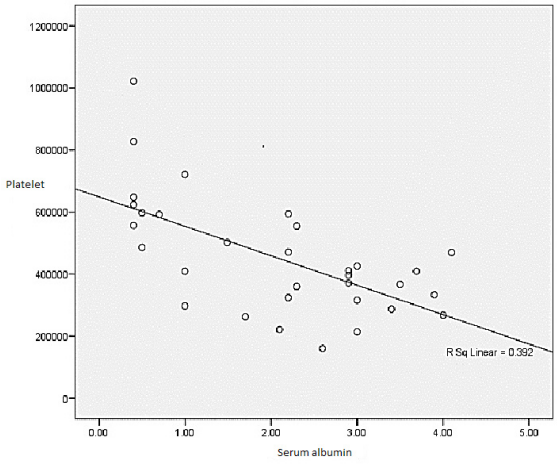

Figure 1. Correlation between serum albumin level and platelet count in pediatric NS patients

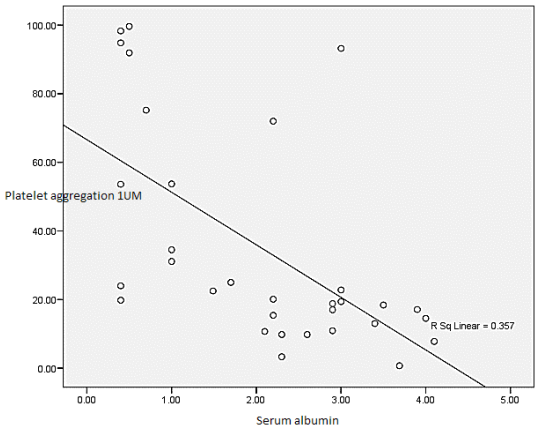

Figure 2. Correlation between serum albumin level and platelet aggregation value, assessed using ADP concentration of 1 µM in pediatric NS patients

consistent with the theory that NS is more commonly found among school children and teenagers. Most of our subjects were diagnosed with steroid-resistant NS, which suggests that Dr. Hasan Sadikin General Hospital status is a top referral hospital.

Decreased serum albumin level is one of laboratory criteria needed to establish a diagnosis of nephrotic syndrome according the *International Study of Kidney Disease in Children* (ISKDC), with cutoff point of 2.5 g/dL.[13] All subjects had at least +2 proteinuria on three urinalyses, based on urine dipstick measurement. Those with marked hypoalbuminemia were noted.

Several previous studies found the high platelet count among NS patients with high platelet count.[14-17] Our subjects had a high mean platelet count of 453,062.50 (SD 187,443.90)/mm^3, ranging between 160,000–1,022,000/mm^3, while 25% of patients had normal platelet count. An Indian study stated that thrombocytosis was always happened among NS patients, with mean (SD) platelet level of 307,000 (49,099.5)/mm^3.[7] Mitall *et al.* found that platelet counts in steroid-sensitive NS and focal segmental glomerulosclerosis (FSGS) NS patients were higher than the control. Similarly, our subjects' mean platelet count was markedly high.

All subjects had received steroid medication, with a range of 1 day to 9 years, none of whom reported remission. Platelet count during remission and after cessation of therapy remained high, but returned to the normal range after long-term remission.[16] The mechanism of elevated platelet count in NS patients is unclear, but several hypotheses suggest links to hypoalbuminemia, hypovolemia, and hypercholesterolemia.[18]

We noted hyperaggregation of platelet in our subjects. An Indian study in 2013 showed increased serum levels of collagen, ristocetin, arachidonic acid, and platelet aggregation rate as assessed by 5 and 2.5 μM ADP concentration tests in 29 pediatric NS patients.[7] Patients with NS experience arachidonic acid (AA) release, which metabolizes into thromboxan A2, in turn inducing hyperaggregation of platelets. This rising aggregation has been connected to hypoalbuminemia, since albumin may inhibit platelet aggregation induced by arachidonic acid and its conversion.

Statistical analysis revealed that serum albumin level correlated in a linear manner with platelet count (correlation coefficient 0.641; P<0.001). Furthermore, statistical analysis of serum albumin level and platelet aggregation value with ADP concentration of 1 μM revealed a moderate coefficient (r=-0.634; P<0.001). Hence, serum albumin had negative correlations with those two variables. A previous retrospective study showed that NS patients with proteinuria and hypoalbuminemia had 8 times higher risk of a venous thromboembolic event.[19] Another study on NS patients also reported that serum albumin level of 3-3.99 g/dL would increase the thromboembolic event

risk by 1.5 times, while serum albumin level of 2.5-2.99 g/dL would increase the thromboembolic event risk by 2.2 times, and serum albumin level below 2.5 g/dL would increase the risk by 2.79 times.[11]

This study had several limitations. The sample size was considered small for assessing correlations between these variables. We also did not analyze other factors that might have contributed to thromboembolic events, such as anticoagulation factors, fibrinolytic factors, factors V, VII, VIII, and X, von Willebrand factor, and total serum protein level. Further studies should be conducted with a larger sample size and include an evaluation of a practical diagnostic value of albumin for thromboembolic events.

In conclusion, serum albumin level negatively correlates with platelet count and platelet aggregation among pediatric NS patients.

Conflict of Interest

Authors stated that there was no competing interest in this study.

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Original Article

Overweight, hypertension and microalbuminuria in urban and rural Bangladeshi schoolchildren

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Abstract

Background The prevalence of childhood overweight and obesity has increased over the last two decades due to high caloric intake and decreased physical activity which occurred in conjunction with increasing prevalence of hypertension. Microalbuminuria is an early sign of damage to the kidney and cardiovascular system. Hypertensive, overweight children have an increased chance of microalbuminuria.

Objectives To assess the prevalence of overweight, hypertension, and microalbuminuria in urban and rural school going children and contributing risk factors and associations.

Methods This cross-sectional study was done in schoolchildren aged 6 to 16 years, from urban and rural areas, in Bangladesh, from September 2015 untill August 2016. Subjects' weights, heights, and blood pressures (BP) were measured. Overweight and hypertension (HTN) statuses were determined with age-appropriate standardized charts. Subjects were divided into overweight and normoweight groups to evaluate risk factors for overweight such as family history (F/H) of obesity, F/H of HTN, daily physical outdoor activities, and monthly family income by comparative analysis. All overweight children were divided into hypertensive and normotensive groups in order to compare their fasting lipid profiles, urine microalbumin, serum creatinine, and random blood sugar.

Results A total of 976 schoolchildren from urban (471, 48.3%) and rural (505, 51.7%) areas were included. Overweight was observed in 22.3% of the urban group and in 8.1% of the rural group. Hypertension was observed in 24.7% of overweight children and in 2.5% of normal weight children. Contributing risk factors for overweight were less physical outdoor activities, F/H of obesity, F/H of HTN, and higher family income. Microalbuminuria and random blood sugar were significantly increased in the overweight with hypertension group compared to the normotensive group.

Conclusion Overweight is a health problem, noted especially in urban areas. Hypertension is also significantly increased in overweight children. Factors like F/H of hypertension, obesity, sedentary lifestyle, and higher socioeconomic status are significantly associated with overweight. Microalbuminuria and increased random blood sugar are also significantly higher observed in hypertensive overweight children compared to normotensive overweight children. **[Paediatr Indones. 2019;59:18-26; doi: http://dx.doi.org/10.14238/pi59.1.2019.18-26].**

Keywords: *microalbuminuria; overweight; hypertension*

Overweight and high blood pressure (HBP) are risk factors directly associated with morbidity and mortality from cardiovascular and renal diseases.[1] An estimated one-third of pediatric arterial hypertension cases are associated with obesity.[2] Overweight was associated with increased sympathetic activities, leptin, and angiotensinogen protein expressed in and secreted by adipose tissue.[3]

Hypertension is an important public health problem in different regions of the world because

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of its high prevalence and concomitant risks of cardiovascular and renal diseases.[4] The prevalence of childhood obesity has increased over the last two decades due to high caloric intake and decreased physical activity.[5] An increased epidemiological shift from secondary hypertension (most often caused by renal disease) to primary (essential hypertension) is the main cause of hypertension in children. Pediatric primary hypertension has become increasingly common in association with other cardiovascular risk factors, including overweight, insulin resistance and dyslipidemia.[6]

Longitudinal epidemiological studies have demonstrated that body mass index (BMI) above the 85th percentile increases the chances for developing hypertension in adolescents and children.[7] An analysis of worldwide data estimated that the total population with hypertension in 2000 was 972 million, with 333 million in countries with established market economies and 639 million in economically developing countries. This number was predicted to increase by 60% (1.56 billion) by 2025.[8] The magnitude of the hypertension burden is predictive of a worldwide cardiovascular disease epidemic.[9] Increasing prevalence of hypertension occurred in conjunction with an increase in the prevalence of overweight. The prevalence of overweight among children and adolescents increased by 2.5% from 1990-2010. At the current rate of increase, the worldwide prevalence is expected to exceed 9% by 2020.[10] An alarming rise in obesity observed across Europe.[11] In England, 66% of men and 55% of women were deemed either overweight or obese.[12]

In Shangdon, China the prevalences of HBP in overweight and normoweight children were 38.7% and 11.4%, respectively.[13] The Bogalusa Heart Study reported that overweight children were 4.5 and 2.4 times likely to have elevated systolic and diastolic blood pressure (SBP, DBP), respectively.[14] Another study in Chinese children and adolescents showed that obese children had 2.9 times higher risk of developing hypertension compared to normoweight children.[15] In addition, waist circumference (WC) and BMI were significantly associated with systolic and DBP in adolescents.[16] An Indian study showed hypertension prevalences of 7.0% in urban and 2.6% in rural adolescents as well as significantly increased prevalence of hypertension with increased BMI.[17] Body mass index was found to be positively related to the risk of high SBP and DBP, with 1.9 times increased risk of high SBP and 1.2 times increased risk of high DBP in those who were either overweight or obese compared to normoweight subjects.[18]

Microalbuminuria is an early sign of damage to the kidney and cardiovascular system.[19] The prevalence of microalbuminuria was reportedly 10-15% in the general population,[20] and 33.2% in schoolchildren. It was significantly higher in females (45.3%), obese subjects (35.4%), and those with hypertension (70.6%).[21] The prevalences of overweight, obesity, and hypertension were 9.3%, 8.9%, and 6.1%, respectively, in schoolchildren aged 5-15 years. Obese children had the highest rate of hypertension, with significantly higher systolic and diastolic BP *z*-scores. The estimated glomerular filtration rates (eGFR) were lower in obese children compared to normoweight children, but the difference was not statistically significant.[22]

In our study, we had several aims: (1) to determine the prevalence of microalbuminuria in schoolchildren with overweight-related hypertension, (2) to determine the prevalence of overweight in both urban and rural schoolchildren, (3) to determine the prevalences of overweight by age groups and sex in schoolchildren, (4) to assess for an association between overweight and HTN in schoolchildren, (5) to identify contributing risk factors for overweight such as family history of obesity, HTN, daily physical outdoor activities, and monthly family income, (6) to determine the prevalence of microalbuminuria in overweight urban and rural schoolchildren, (7) to determine the prevalence of microalbuminuria in overweight boys *vs.* girls, (8) to perform a comparative analysis between overweight children with and without hypertension by lipid profiles, random blood sugar (RBS), serum creatinine, and urinary microalbumin.

Methods

This cross-sectional descriptive study was done in both rural (Gazaria, Munshigonj) and urban (Narayangonj City) primary and secondary schools in Bangladesh, from September 2015 to August 2016. The inclusion criteria were healthy schoolchildren aged 6 to 16 years. The exclusion criteria were children with

overweight and hypertension due to secondary causes (renal, endocrine, cardiovascular, or drugs) by history and clinical examination, children who refused to participate, or children outside the age range of 6-16 years.

The sample was collected from the following schools: Agrani Biddaniketon School, Narayangonj (urban); and Daulatpur Bright Preparatory School, Daulat pur, Rasulpur Model Primary School, and Gazaria Pilot High School, Gazaria, Munshiganj (rural), by a convenient purposive sampling method. Urban was defined as area under city corporation or municipality in Bangladesh, while rural was area other than a city corporation or municipality in Bangladesh.

Before collecting data, permission was granted by the schools' headmasters. Written informed consent was signed either by parents or legal guardians. Data were collected by a structured questionnaire that included all variables of interest.

Anthropometric measurements including height and weight were measured following standardized procedures. Weight was measured to the nearest 0.1 kg with a portable digital bathroom scale. Height was measured to the nearest 0.1 cm using a stadiometer with the subject barefooted and his/her back against the wall. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters, and categorized as normal weight (BMI between 5th to 85th percentile), underweight (BMI < 5th percentile), or overweight (BMI >85th percentile), according to the chart.[23]

Blood pressure was measured by the auscultatory method with an aneroid sphygmomanometer, with the subject's right arm at heart level, using a pediatric cuff of appropriate size at basal state. The cuff should cover at least two-thirds of the arm. Blood pressure was measured 3 times before a hypertensive diagnosis was given. The subject was seated quietly for at least 5 minutes before measurement. Systolic BP was determined in Korotkoff's phase I, whereas diastolic BP was determined in Korotkoff's phase V. The lowest values for SBP and DBP were used in the analyses. Blood pressure was categorised as hypertension (SBP and/or DBP values >95th percentile) or normotensive (SBP and/or DBP values between 5th and 95th percentile), according to sex, age, and height.[24]

Overweight children underwent further evaluations of daily physical outdoor activities, monthly family income, family history of obesity and hypertension, as well as evidence of renal, endocrine and cardiovascular diseases by history and clinically. We also measured overweight subjects' lipid profile, urine microalbumin, serum creatinine, and RBS (*Dimension RXL Max Machine-2*, Biochemistry Department, BSMMU, Dhaka and *Popular Laboratory and Diagnostic Center*, Narayangonj). Microalbuminuria was defined as an increased urine albumin excretion (30-300 mg/24 h) that is undectable by standard protein dipstick testing.[25] In this study, urine microalbumin >20 mg/L was considered to be microalbuminuria.

The frequency of overweight, normal weight, underweight, and HTN were estimated as percentages in both urban and rural groups. Comparative analysis of normal weight and overweight in urban and rural groups was done by Chi-square test. The percentage of HTN was estimated and comparatively analyzed in the overweight and normoweight groups. Comparative analyses were done between the overweight and normoweight group to assess for possible contributing risk factors for overweight such F/H of obesity, F/H of HTN, daily physical outdoor activities, and monthly family income. Lastly, the fasting lipid profile, urine microalbumin, serum creatinine, and RBS were compared in overweight children with and without hypertension group.

We used *SPSS software version 22* for statistical analyses. For all statistical tests, P values <0.05 were considered to be statistically significant. Continuous variables are presented as mean (SD). Continuous variables were compared by student's unpaired T-test, and categorical variables by Chi-square test. Prior to the commencement of this study, the thesis protocol was approved by the Institutional Review Board of BSMMU, Dhaka.

Results

Table 1 shows the demographic characteristics of participants. Among them, 471 (48.3%) were from urban and 505 (51.7%) were from rural areas. Among urban participants, 257 (54.6%) were boys and 214 (45.4%) were girls, similar to the rural distribution of 299 (59.2%) boys and 206 (40.8%) girls. Among urban participants, 141 (29.9%) were aged 6-10 years and 330

(70.1%) were 11-16 years; among rural participants, 240 (47.6%) were 6–10 years and 265 (52.4%) 11-16 years, which were significantly different (P=0.001). The prevalences of overweight were also significantly different, with 105 (22.3%) in urban and 41 (8.1%) in rural children. Likewise, the prevalence of HTN was significantly higher in urban (38 subjects, 8.1%) than in rural children (15 subjects, 3.0%). Overweight with hypertension was seen in 5.7% and 1.8%, of urban and rural, respectively. Overweight, HTN, and overweight/HTN combined were all significantly higher in urban than rural subjects (P<0.001 for all).

Table 2 shows the prevalences of overweight among urban and rural boys and girls. Overweight among boys was highest (33.7%) in the 12-14-year age group, followed by 22%, 20.7%, 13%, and 10.4% in the age groups of 10–12 years, 6-8 years, 14-16 years, and 8-10 years, respectively. For girls, overweight was highest (29%) in the 10-12-year age group, followed by 23.2%, 18.8%, 16.0%, and 13% in the age groups of 6-8 years, 12-14 years, 8-10 years, and 14-16 years, respectively. The only significant difference between boy and girls was in the 12-14 year age group, with more overweight boys than overweight girls (P=0.042).

Table 3 shows the frequency of BMI status and the frequency of HTN in urban and rural children by BMI status. Of 471 urban subjects, 22.3% were overweight, 65.8% were normal weight, and 11.9% were underweight. Hypertension was seen in 25.7% of the overweight and 3.5% of the normoweight urban subjects. In rural areas, 8.1% were overweight, 71.5% normoweight, and 20.4% underweight; HTN was seen in 21.9% of the overweight group and 1.6% of the normoweight group.

Table 4 shows the association between BMI status and BP status. Significantly more overweight subjects were hypertensive (36; 24.7%) than normoweight subjects (17; 2.5%) (P<0.001). **Table 5** shows the associations between BMI status and SBP or DBP. SBP and DBP were both significantly higher in overweight than in normoweight children. **Table 6** shows the association between monthly family income and BMI status. Overweight was associated with the high income group. The majority of overweight and normoweight subjects were in the 10,000-30,000 Tk/mo group. Significantly more normoweight than overweight subjects were in this income

Table 1. Demographic characteristics of participants by location (n=976)

Characteristics	Urban (n=471)	Rural (n=505)	P value
Sex, n (%)			
Male	257 (54.6)	299 (59.2)	0.143
Female	214 (45.4)	206(40.8)	
Age group, n (%)			
6 -10 years	141 (29.9)	240 (47.6)	0.001
11-16 years	330 (70.1)	265 (52.4)	
Overweight, n (%)	105 (22.3)	41 (8.1)	<0.001
HTN, n (%)	38 (8.1)	15 (3.0)	<0.001
Overweight +HTN, n (%)	27 (5.7)	9 (1.8)	<0.001

Chi-square test

Table 2. Overweight among boys and girls by age grouping

Age	Boys, n(%) (n=77)	Girls, n(%) (n=69)	P value
6-8 years	16 (20.7)	16 (23.2)	0.725
8-10 years	8 (10.4)	11 (16)	0.319
10-12 years	17 (22)	20 (29)	0.338
12-14 years	26 (33.7)	13 (18.8)	0.042
14-16 years	10 (13)	9 (13)	0.992

Table 3. BMI category and HTN in urban subjects (n=471)

BMI status	Urban		Rural	
	Urban, n (% of urban subjects)	HTN and urban, n (% of BMI category)	Rural, n (% of rural subjects)	HTN and rural, n (% of BMI category)
Overweight	27 (25.7)	41 (8.1)	9 (21.9)	105 (22.3)
Normoweight	310 (65.8)	11 (3.5)	361 (71.5)	6 (1.6)
Underweight	56 (11.9)	0 (0)	103 (20.4)	0 (0.0)

category [547 (81.5%) vs. 65 (44.5%), respectively; P<0.001)]. However, significantly higher percentages of overweight subjects were in the 30,000-100,000 Tk/mo and >100,000 Tk/mo categories (P<0.001 for both).

Table 7 shows the relationship between physical outdoor activities and family history of obesity/ hypertension with BMI status. The frequency of overweight was significantly higher among the children who engaged in physical outdoor activities for <45 minutes/day than in the ≥ 45 minutes/day group (P<0.001). Significantly higher percentages of overweight children had F/H of obesity as well as F/H of HTN, compared to the normoweight subjects (P<0.001 for both).

Table 8 shows that the percentages of microalbuminuria in overweight children were not significantly different in urban and rural subjects (13.3% vs. 17.1%; P=0.824). There was no significant difference in microalbuminuria in overweight boys and girls [14.2% vs. 14.4%, respectively; (P=0.982)].

Table 9 shows the comparison of biochemical parameters (lipid profiles, creatinine, RBS, and urinary microalbumin) in overweight children with HTN and all overweight subjects. Dyslipidemia and high serum creatinine were higher in the hypertensive than the normotensive group, but the difference was not statistically significant. In contrast, urinary microalbumin and RBS level were significantly higher in the hypertensive than the normotensive group (P=0.001 and P=0.004, respectively).

Table 4. Analysis of overweight and HTN

BP status, n (%)	Overweight (n=146)	Normoweight (n=671)	Total (n=817)	P value
Hypertensive	36 (24.7)	17 (2.5)	53 (6.5)	<0.001
Normotensive	110 (75.3)	654 (97.5)	764 (93.5)	<0.001

Table 5. Association of blood pressures with BMI

BMI	SBP, mmHg		DBP, mmHg	
	Mean (SD)	95% CI	Mean (SD)	95% CI
Overweight (n=146)	112.6 (14.6)	109.7 to 115.4	74.7 (9.7)	72.8 to 76.6
Normoweight (n=671)	100.5 (11.8)	99.2 to 101.8	67.1 (8.7)	66.1 to 68.1
P value	<0.001		<0.001	

Unpaired T-test

Table 6. Association of monthly family income with BMI

Monthly family income, n (%)	Overweight (n=146)	Normoweight (n=671)	P value
< 10,000 Tk	13 (8.9)	27 (4)	<0.054
10,000 – 30,000 Tk	65 (44.5)	547 (81.5)	<0.001
30,000 – 100,000 Tk	46 (31.5)	92 (13.7)	<0.001
>100,000 Tk	22 (15.1)	5 (0.7)	<0.001

Chi-square test, Tk=Bangladesh taka

Table 7. The relationship between some parameters with BMI status

Parameters, n(%) / BMI status, n(%)	Physical outdoor activities			Family history (F/H)		
	<45 min/day (n=160)	≥45 min/day (n=657)	P value	F/H of obesity	F/H of HTN	P value
Overweight	136 (85.0)	10 (1.5)	<0.001	71 (48.6)	69 (47.3)	<0.001
Nomoweight	24 (15.0)	647 (98.5)		12 (1.8)	18 (2.7)	

Chi-square test

Table 8. Microalbuminuria (>20 mg/L) in correlation with residency and gender

	Microalbuminuria	P value
Residency, n(%)		0.824
Urban (n=105)	14 (13.3)	
Rural (n=41)	7 (17.1)	
Total (N=146)	21 (14.3)	
Sex		
Boys (n=77)	11 (14.2)	0.982
Girls (n=69)	10 (14.4)	
Total (N=146)	21 (14.3)	

Chi-square test

found that in England, 66% of men and 55% of women were either overweight or obese.[12] In our study, the largest groups of overweight subjects were the 12-14 years group (33.7%) of boys, and the 10-12 years group in girls (29%). Hormonal effect at pubertal ages may contribute. Adrogue et al. found that the prevalence of HTN also increased progressively with age.[26]

In our study, the prevalence of HTN was 8% in urban and 2.9% in rural children. In comparison with

Table 9. Comparison of mean lipid profiles, serum creatinine, urinary microalbumin, and RBS in overweight subjects with hypertension and without hypertension

	Overweight with HTN (n=36)	95% CI	Overweight without HTN (n=110)	95% CI	P value
Mean cholesterol (SD), mg/dL	153.8 (40.4)	142.6 to 164.9	145.5 (35.4)	138.2 to 152.8	0.198
Mean HDL (SD), mg/dL	30.1 (8.2)	27.8 to 32.3	30.1 (8.9)	28.3 to 31.9	0.994
Mean LDL (SD), mg/dL	97.1 (32.5)	88.1 to 106.1	87.7 (25.3)	82.5 to 92.9	0.053
Mean triglycerides (SD), mg/dL	151.4 (26.3)	144.1 to 158.6	145.1 (25.1)	139.9 to 150.3	0.153
Mean creatinine (SD), mg/dL	0.67 (0.55)	0.55 to 0.78	0.64 (0.11)	0.61 to 0.67	0.749
Mean urinary microalbumin (SD), mg/L	14.8 (12.3)	11.4 to 18.2	9.7 (5.5)	8.6 to 10.8	0.001
Mean RBS (SD), mmol/L	5.8 (1.2)	5.5 to 6.0	5.3 (0.5)	5.2 to 5.4	0.004

Unpaired T-test

Table 10. Comparison of microalbuminuria in overweight children with and without HTN

Urinary microalbumin	Overweight + HTN, n (%) (n=36)	Overweight without HTN, n (%) (n=110)	P value
>20 mg/L	15 (41.6)	6 (5.4)	0.001
≤20 mg/L	21 (58.4)	104 (94.6)	

Table 10 shows the comparison of urinary microalbumin in overweight children with and without hypertension. High urinary microalbumin (>20 mg/L) was observed in hypertensive group (41.6%) and it was low in overweight children without hypertension group (5.4%).

Discussion

In this cross-sectional study, we examined the prevalence of overweight and its relationship with BP in urban and rural schoolchildren aged 6 to 16 years. Of 976 participants, 15% were overweight, 22.3% of urban and 8.1% of rural children. Aucott *et al.* found an alarming rise in obesity in the US in the last 15 years, with one-third of the population affected.[11] Similar trends were seen across Europe. Avenell *et al.* normoweight children, those who were overweight had significantly higher proportion of high BP. High BP was found in 24.7% of the overweight group and 2.5% of the normoweight group. These results were similar to those of other studies in different countries. Mohan *et al.* in India found that the prevalence of hypertension was about 7.0% and 2.6% amongst urban and rural adolescents, respectively. They also found a significant increase in prevalence of hypertension with an increased BMI.[17] Muntner *et al.* found that the childhood obesity epidemic was associated with the increasing prevalence of HTN.[27] Duzova *et al.* noted that the prevalences of overweight, obesity, and hypertension were 9.3, 8.9, and 6.1%, respectively, in schoolchildren aged 5-15years. Obese children had the highest rate of hypertension. Systolic and diastolic BP z-scores were significantly higher in obese children.[22] Din-Dzietham et al. assessed secular high

BP trends in children and adolescents enrolled in US national surveys, and concluded that the increase was largely attributable to the increase in obesity.[28] Sorof found that the prevalence of HTN in was 1 to 22%.[29] Many of these studies also documented an association between HTN and obesity. In Shangdon, China, Jing Dong *et al.* found that the prevalence of high BP in obese and normoweight children were 38.7% and 11.4%, respectively.[13] The prevalence of HTN seems to be increasing in association with BMI in Canada as well. In our overweight subjects, F/H of obesity was present in 48.6% and F/H of HTN was present in 47.3%. Similarly, Jung *et al.* found that a family history of HTN was present in approximately 50% of hypertensive children.[30] Likewise, Stamler et al. and Longini et al. found that HTN had long been known to cluster within families, possibly due to shared environmental exposures (obesity, salt intake, lifestyle, etc.).[31,32] We also found that 93.2% of the overweight group compared to 3.6% of the normoweight group engaged in <45 minutes of daily physical outdoor activities. Pardee et al. found that in obese children, the amount of time spent watching TV was associated with both HTN and the severity of obesity.[33] Cleroux et al. found that moderate activity was more effective than vigorous activity in reducing SBP.[34]

We analyzed the overweight children in terms of lipid profile, serum creatinine, RBS, and urinary microalbumin between hypertensive and normotensive children. Hypertension was strongly associated with high urinary microalbumin and increased blood glucose level. These conditions may be due to early kidney damage and insulin resistance. Serum creatinine and dyslipidemia were higher in the hypertensive group, but not significantly. Duzova *et al.* also observed that mean estimated GFR was lower in obese children, but not of statistical significance. No study has shown dyslipidemia in obese children.[22]

In our study, microalbuminuria (>20mg/L) was seen in 14.3% of overweight children overall, with 13.3% urban and 17% rural, 14.2% boys and 14.4% girls, 5.4% normotensive, and 41.6% hypertensive. Okpere *et al.* reported that the prevalence of microalbuminuria in school children was 33.2%. It was significantly higher in females (45.3%), obese subjects (35.4%), and those with hypertension (70.6%).[21] Mogenson et al. found that the prevalence of microalbuminuria in the general population was 10-15%.[20]

Diercks *et al.* found that microalbuminuria was an early sign of damage to the kidney and cardiovascular system.[19] Assadi noted a relationship between left ventricular hypertrophy (LVH) and microalbuminuria in hypertensive subjects, and documented that urinary albumin excretion was increased in children and adolescents with HTN in correlation to LVH.[35] Nguyen *et al.* found that microalbuminuria was associated with HTN among overweight adolescents.[36] Lubrano *et al.* found that children with prehypertension showed increased prevalence of microalbuminuria and proteinuria, while children with white coat hypertension showed no signs of HTN-related renal damage.[37]

Our study limitations were small sample size, no comparison of lipid profile, blood sugar, urine microalbumin and serum creatinine with normal weight and underweight children due to social and financial constraints, and secondary causes of overweight and HTN was excluded only by history and clinically. We recommend to conduct a larger multicenter study with a large sample size which provide a comparison of all parameters with normal and underweight children.

Overweight is a health problem especially in urban areas. Hypertension is common in overweight children. Factors like family history of hypertension and obesity, sedentary life style, better solvency are linked to overweight. Microalbuminuria and increased random blood sugar are significantly associated with overweight hypertensive children. Overweight children may have hypertension, as well as microalbuminuria, which can cause renal insufficiency in the future.

Conflict of Interest

None declared.

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Original Article

Intrinsic risk factors for gross motor delay in children aged 6-24 months

Joanna Erin Hanrahan, Irawan Mangunatmadja

Abstract

Background Gross motor is one of the skill domain with the highest parental concern as mastering it determines the autonomy of a child. Several internal risk factors including perinatal asphyxia, prematurity, low birth weight, wide fontanelle, and microcephaly have been studied in predicting gross motor delay with varied results. This study is made to arrange a strategic intervention on the prevention of delayed development.

Objective To evaluate perinatal asphyxia, gestation age <37 weeks, birth weight <2500 grams, microcephaly, and wide fontanelle as predictors of gross motor delay in children aged 6-24 months.

Methods A case control study design was used. Data collection was conducted by direct assessment of gross motor skill and parents' interview in Cipto Mangunkusumo National Hospital and Anakku Clinic, South Jakarta. Children with gross motor delay were included in the case group and children with normal gross motor were included in the control group. Data was analyzed using bivariate and multivariate analysis with a statistical significance value of P<0.05 and 95% confidence intervals.

Results One hundred and twenty-six subjects were studied, with 63 children in the case group and 63 children in the control group. Baseline characteristics of subjects were similar between the two groups. Microcephaly and gestation age <37 weeks were predictors of gross motor delay [(aOR 4.613; 95%CI 2.023 to 10.521; P<0.001) and (aOR 3.668; 95%CI 1.153 to 11.673; P=0.028)], respectively.

Conclusion Microcephaly and gestation age <37 weeks are significant predictors of gross motor delay in children aged 6-24 months. **[Paediatr Indones. 2019;59:27-32; doi: http://dx.doi.org/10.14238/pi59.1.2019.27-32].**

Keywords: *gross motor delay; risk factor; 6-24 month old*

There are 4 domains of development that has to be accomplished by a child according to his age range, such as gross motor, fine motor, communication and language, and cognitive. If a child fails to master a skill according to his age group, he is said to have a delayed development. Developmental delay can occur in those 4 domains, including gross motor.[1] Gross motor represents the role of big muscles that are responsible in movements such as walking, running, and jumping. The development of a child is like a mile stone. To be able to reach the next skill, a child has to mastered the skill in the lower stage. Gross motor is the first domain of the milestone that has to be mastered by a child.[2] Gross motor helps children to interact with their environment thus giving them chance to maximize their potential in other domains of development. Therefore, if a child has gross motor delay, he is in higher risk of having developmental delay in the other 4 domains.[3]

Global data shows that 5-10% of the children population have delayed development. This data also reports that the most frequent developmental delay

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occur in gross motor domain.[4] Unfortunately, out of all the children with motoric delay, only 10% of those who were succeed in receiving early intervention while early intervention itself is a strategic prevention in preventing developmental delay. Determining the risk factors of gross motor delay is seen to have the potential in increasing the proportion of children who received early intervention. Besides, if the detection of this developmental delay is too late, a child will lose his golden period of development.[3]

Several internal risk factors have been studied in predicting gross motor delay yet with varied results. Moreover, there has not been any study on internal risk factors of predicting gross motor delay in Indonesia. Therefore, this study is made to evaluate the internal risk factors, such as perinatal asphyxia, birth weight <2500 grams, gestation age <37 weeks, microcephaly, and wide fontanelle on predicting gross motor delay. The study is done in children aged 6 to 24 months. The lowest age cut off, which is 6 months, is chosen because this is the time when gross motor skill can be first assessed clearly and the highest age cut off, which is 24 months, is chosen referring to 3 years of age as the maximum golden period of a child's brain development, therefore we provide a year spare time for stimulation and catching-up the developmental delay.[5]

Methods

A case-control study was conducted in children aged 6-24 months in Paediatric Polyclinic of Cipto Mangunkusumo National Hospital and Anakku Clinic, South Jakarta from February 2018 to July 2018. Subjects were recruited consecutively. Children aged 6-24 months with gross motor delay whose parent had agreed to sign the informed consent form were included in the case group. As for the control group, we included all children aged 6-24 months with normal gross motor whose parent also had agreed to sign the informed consent form. Data were collected from direct assessment of gross motor skill and parents' interview regarding their child's history. We first identified subjects with gross motor delay and without gross motor delay, then we collected the data of the possible internal risk factors such as history of perinatal asphyxia, birth weight, head circumference, gestation age of the child before he was born, and size of the fontanelle retrospectively.

In this study, gross motor delay was assessed using the Developmental Milestone Table according to the children's age that can be seen in **Table 1**.[6] Children who were not able to do the gross motor skill associated to the group age below their age, were said to have gross motor delay. For example, if a nine-month-old was unable to sit on their own, which was a skill that has to be mastered in a six-month-old (**Table 1**), he was said to have gross motor delay. Whereas if this child was able to sit on his own, yet was not able to pulled to stand, he was still said to have normal gross motor. Using the definition published by WHO, low birth weight was defined as birth weight less than 2500 grams.[7] Also using the definition published by WHO, prematurity was defined as gestation age less than thirty-seven weeks.[8] Head circumference of the child was measured using plastic tape and the result was plotted to Nelhaus graph. Microcephaly was defined when a value was found below the SD -2 curve.[9] Information about history of perinatal asphyxia was obtained through parents' interview. The child was said to have a history of perinatal asphyxia when the parents said there was no direct crying when the child was born. In this study, we measured the size of anterior fontanelle to define the fontanelle size as it is the last fontanelle that will be closed in a child development. The measurement was done by measuring the horizontal and vertical axis of the fontanelle, then the sum of these was divided by two. The reference used to define the recommended size of anterior fontanelle according to the child's age was a study by Esmaeili *et al.*[10]

Table 1. Cut-off points for age of attainment of developmental milestones[6]

Age (months)	Gross motor development
3	Lift up on hands, no head lag if pulled to sit from supine
6	Sits without support
9	Pulled to stand
12	Walks alone
18	Runs
24	Walks up and down stairs

Unpaired case control method was used to calculate the required sample size, with an assumed

odds ratio (OR) for each variable (perinatal asphyxia, birth weight, head circumference, gestation age, fontanelle size). We assumed the largest OR for fontanelle size, with a power of 80% and a type I error of 5%, resulting in 63 subjects in each group without matching. The chi-square test was used in bivariate analysis. Variables with a P value of <0.25 in bivariate analysis were included into the multivariate analysis. Logistic regression with backward stepwise elimination was used in multivariate analysis. Results were presented in OR, 95% confidence intervals, and a statistical significance value of P. All data were analyzed by *SPSS for Mac 23.0*. The study protocol was approved by the Medical Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia.

Results

There were 63 subjects in the case group (gross motor delay) and 63 subjects in the control group (normal gross motor). Nineteen subjects from the case group and 35 subjects from the control group were obtained from Klinik Anakku, South Jakarta. The rest were obtained from Paediatric Polyclinic of Cipto Mangunkusumo National Hospital. Both groups were similar in their demographic characteristics shown in **Table 2**.

Predictive factors for gross motor delay in children are shown in **Table 3**. Bivariate analysis showed that perinatal asphyxia, gestation age <37 weeks, birth weight <2500 grams, and microcephaly were all significant predictive factors for gross motor delay. Multivariate analysis showed that microcephaly (aOR 4.613; 95% CI 2.023 to 10.521; P<0.001) and gestation age <37 weeks (aOR 3.668; 95% CI 1.153 to 11.673; P=0.028) were significant predictive factors for gross motor delay. The result of multivariate analysis can be seen in **Table 4**.

Discussion

After taking into account 4 predictive factors (perinatal asphyxia, prematurity, low birth weight, and microcephaly) in multivariate analysis, our results showed that microcephaly and prematurity (gestation age <37 weeks) were significant predictors of gross motor delay. It was found in our study that children with microcephaly had higher odds of developing gross motor delay compared to those without microcephaly. This was consistent with several previous studies, including a study held by Scharf RJ *et al.*,[12] who assessed the head circumference of children when they were 9 months and 24 months old, where they

Table 2. Baseline characteristics of subjects

Characteristics	Gross motor delay (n=63)	Normal gross motor (n=63)	Total (%)
Gender, n(%)			
Male	41 (65.1)	38 (60.3)	79 (62.7)
Female	22 (34.9)	25 (39.7)	47 (37.3)
Age			
< 1 year old	23 (36.5)	17 (27)	40 (31.7)
1-2 years old	40 (63.5)	46 (73)	86 (68.3)
Gestational age			
< 37 weeks	16 (25.4)	5 (7.9)	21 (16.7)
≥ 37 weeks	47 (74.6)	58 (92.1)	105 (83.3)
Birth weight			
< 2500 gram	17 (27)	6 (9.5)	23 (18.3)
≥ 2500 gram	46 (73)	57 (90.5)	103 (81.7)
Microcephaly			
Yes	36 (57.1)	13 (20.6)	49 (38.9)
No	27 (42.9)	50 (79.4)	77 (61.1)
Wide fontanelle			
Yes	25 (39.7)	20 (31.7)	45 (35.7)
No	38 (60.3)	43 (68.3)	81 (64.3)

Table 3. Bivariate analysis of predictive factors of gross motor delay (n=126)

Variables	Gross motor delay (n=63)	Normal gross motor (n=63)	OR (95% CI)	P value
Perinatal asphyxia				
Yes	14	3	5.714	0.004
No	49	60	(1.553 to 21.026)	
Gestation age				
< 37 weeks	16	5	3.949	0.009
≥ 37 weeks	47	58	(1.347 to 11.574)	
Birth weight				
< 2500 grams	17	6	3.511	0.011
≥2500 grams	46	57	(1.281 to 9.625)	
Microcephaly				
Yes	36	13	5.128	<0.001
No	27	50	(2.332 to 11.280)	
Wide fontanelle				
Yes	25	20	1.414	0.353
No	38	43	(0.680 to 2.942)	

Table 4. Multivariate analysis with backward stepwise elimination of predictive factors of gross motor delay

Variables	B	SE	Adjusted OR (95%CI)	P value
Gestation age <37 weeks	1.300	0.591	3.668 (1.153 to 11.673)	0.028
Microcephaly	1.529	0.421	4.613 (2.023 to 10.521)	<0.001
Perinatal asphyxia	0.708	0.057	3.849 (0.960 to 15.430)	1.348
Birth weight <2500 grams	0.422	0.680	1.526 (0.402 to 5.785)	0.534

found that children with small head circumference had higher odds to develop gross motor delay with adjusted OR in 9 months was 2.71 (95%CI 1.62 to 4.56) and adjusted OR in 24 months was 3.28 (95%CI 1.61 to 6.67), a study by Gordon-Lipkin *et al.*[11] that showed children with microcephaly had significant increased risk of developing gross motor delay, and by Uswatun *et al.*[9] who also stated that there was a significant association between head circumference and global developmental delay. In the process of growth and development of a child, head circumference is often associated with the size of his brain. Microcephaly showed that there is a disruption in neurodevelopment, hence was not able to support his development, including his motor development.[9]

Our finding about prematurity was also aligned with several previous studies, such as a study by Bang K[14] who reported that prematurity was a significant predictor for delay development and a study by De Moura DR *et al.*[13] that also showed history of prematurity in children was significantly associated with delayed development, especially in motoric and social area. They also reported in their study that gross motor domain was the most domain influenced by history of prematurity. Another previous study by Kerstjens JM *et al.*[15] also showed consistent finding with our result. They found that children who were born prematurely had 1.14 times higher odds in developing gross motor delay every one-week reduction of their gestational age before aterm (OR 1.14; 95%CI 1.09 to 1.19; P<0.001). This study demonstrated that the risk of delayed development will increase exponentially, inversely proportional to the reduction of child's gestational age starting from 25 to 36 weeks. This was supported by the fact that

the optimum growth of the brain happens during the 3rd trimester of pregnancy. In this range of time, cortex volume of the brain was increasing until four times, also followed by increment of synaptogenesis, growth of neurons, myelination, and focused apoptosis, which were all directed to the enhancement of connectivity in the brain. In utero environment was more adequate to support all the brain maturation process compared to post-natal environment. Injury to the brain caused by disturbance of its maturation process was suspected to have a role in increasing the risk of gross motor delay in children with premature history.[15] Nonetheless, a study by Arumsari *et al.*[16] presented contradicted result with our study. They found that after adjusting with other factors, prematurity insignificantly associated to gross motor delay occurrence. It was stated that the insignificance found might be due to the small sample size and short duration of study. However, Arumsari et al. also discussed that global development delay was associated with multi-factors so that a premature child might experience normal development if other factors related to his growth and development were sufficient.[16]

Limitation to this study was biased information which might be obtained from retrieving data retrospectively, especially history of perinatal asphyxia, birth weight, and gestation age of the child. Also, history of perinatal asphyxia was only assessed through parents' interview in asking whether there was a history of direct crying after the child was born.

We finally conclude that microcephaly and gestation age <37 weeks are significant predictive factors for gross motor delay in children aged 6-24 months.

Conflict of Interest

None declared.

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Original Article

Association between low-grade chronic inflammation with adipocytokines and body fat mass in superobese male children

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Abstract

Background Obesity causes adipocytokines dysregulation and enhances the pro-inflammatory response. Low-grade, chronic, inflammation is related to cardiometabolic diseases.

Objective To evaluate the status of low-grade chronic inflammation in pre-pubertal, obese boys and its potential correlation to adipocytokines and body fat mass.

Methods This cross-sectional study included pre-pubertal, male, superobese children as the subjects. We determined obesity status using the CDC 2000 BMI-for-age chart. Body fat percentage was measured using bioelectric impedance analysis (BIA). Fasting blood specimens were collected to evaluate hsCRP, leptin, adiponectin, and TNF-α levels.

Results Eighty subjects were recruited into this study, with a mean age of 6.9 years. Ten subjects (12.5%) had low-grade chronic inflammation (hsCRP level ≥ 1 mg/L). The levels of hsCRP was not correlated with leptin, adiponectin, and TNF-α levels. A weak, but significant correlation was observed between hsCRP level and body fat mass ($r = +0.383$; $P<0.0001$). The hsCRP level increased with increasing body fat mass, until it reached its peak at body fat mass of 28 kg. Beyond that point, hsCRP level was stable.

Conclusion Low-grade chronic inflammation begins at a young age in obese children. The hsCRP level has a weak correlation with body fat mass, but no correlations with adipocytokine levels. Prevention and treatment of childhood obesity should be prioritized to prevent further cardiovascular and metabolic diseases. **[Paediatr Indones. 2019;59:13-7; doi: http://dx.doi.org/10.14238/pi59.1.2019.13-7].**

Keywords: *hsCRP; adipocytokines; body fat mass; superobese; children*

In this century, obesity is one of the most crucial issues in childhood. The prevalence of childhood obesity varies across the world, but it is quite high in Asian countries, including Indonesia.[1,2] In 2013, the prevalence of overweight-obesity among Indonesian children aged 5-12 years was 18.8%.[3]

Obesity causes impairment of energy homeostasis and dysregulation of lipid and carbohydrate metabolism. An excess of these substances may increase mitochondrial activity, including the electron transport chain. Tissue (especially adipose) becomes relatively hypoxic due to an increase in oxygen demand. Local adipose hypoxia causes dysregulation of adipocytokines, generation of reactive oxygen species, and activation of several kinases that are related to the pro-inflammatory response.[4,5]

Interaction between immune homeostasis and metabolism has been observed in many studies. Cytokines released from immune cells may affect the ability of adipocytes to regulate carbohydrate and lipid metabolism.[6,7] Previous studies also found that low-grade chronic inflammation is

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related to cardiometabolic syndrome, including insulin resistance and atherosclerosis.[8] In addition, adipocytokines (adipokines) released from adipocytes may alter immune systems. Adipocytokines also play an important role in energy homeostasis.[6,7]

C-reactive protein (CRP), an acute-phase reactant, is produced by the liver. The CRP level may dramatically increase in the presence of significant inflammation. A more sensitive CRP, high-sensitivity CRP (hsCRP), can be used to predict low-grade inflammation caused by medical conditions.[9]

At present, our knowledge about low-grade chronic inflammation in pre-pubertal obese children is limited. Low-grade chronic inflammation in pre-pubertal children is important because many studies found that the pathogenesis of chronic cardiometabolic disease has developed since a young age. We only focused on male children to rule out the potential effect of sex hormones. In this study, we aimed to determine the status of low-grade chronic inflammation (hsCRP level) in pre-pubertal, obese boys and its potential correlation with adipocytokines and body fat mass.

Methods

This cross-sectional study with pre-pubertal, superobese boys as the subjects, was conducted at Cipto Mangunkusumo Hospital Jakarta, a private hospital at East Jakarta, a private hospital at Bekasi, and a primary school in Central Jakarta. This study was approved by Ethics Committee of the Universitas Indonesia Medical School.

We calculated the minimum required sample size using a formula for numeric-numeric correlation, resulting in a minimum of 38 subjects needed to perform a two-tailed correlation analysis. The inclusion criteria were males, aged 5-9 years, with normal growth and development, and obesity solely caused by excessive food intake, as proven by dietary analysis. The exclusion criteria were acute illness, consumption of medications that affect body weight, obesity caused by endogenous factors (genetic or endocrine disease), and those on a diet program.

Body mass index (BMI) was calculated by dividing body weight (in kilogram) by the square of body height (in meters-squared). Superobese was defined as a BMI > 97th percentile on the *CDC 2000* growth chart.[10] We calculated body fat mass by multiplying body weight by body fat percentage, which was measured by bioelectric impedance analysis (BIA) using a *TANITA Inner Scan Body Composition Monitor type BC-545*, Japan. Subjects' blood specimens for quantifying hsCRP and adipocytokine levels were collected after the subjects had fasted for 12 hours. Adipocytokine (leptin, adiponectin, and TNF-α) levels were examined using ELISA technique. The hsCRP levels were categorized into high (≥1 mg/L) or normal (<1 mg/L).[11]

Data are presented numerically. The correlations between low-grade chronic inflammation with body fat mass and adipocytokines were analyzed using Pearson's correlation, with Spearman's correlation as the alternative. Data analysis was performed using *SPSS program version 20.0* (*IBM*, Chicago, IL, USA).

Results

We recruited a total of 80 pre-pubertal, male children for this study. Mean age of subjects was 6.9 years; and the youngest subject was 5 year old. All subjects were superobese (>97th percentile BMI-for-age CDC chart), with median BMI of 24.35 kg/m2. The subject with the lowest body fat had 22.2% body fat percentage, which was equal to 6.28 kg of body fat mass (**Table 1**). Ten subjects (12.5%) had high hsCRP levels (**Table 2**).

We did not observed any correlations between hsCRP level with leptin, adiponectin, or TNF-α level. However, hsCRP level had a weak, but significant positive correlation with body fat mass ($r=+0.383$; $P<0.0001$) (**Table 3**). A scatter plot of hsCRP and

Table 1. Demographic and anthropometric characteristics of subjects

Characteristics	(N=80)
Mean (SD)age, years	6.91 (1.29)
Mean body weight (SD), kg	41.07 (9.77)
Mean body height (SD), cm	126.65 (9.84)
Median BMI (range), kg/m^2	24.35 (19.1-39.8)
Median body fat percentage (range), %	33.35 (22.2-63.1)
Median body fat mass (range), kg	14.71 (6.28-42.4)

Table 2. High-sensitivity CRP and adipocytokine levels

Variables	(N=80)
Median hsCRP (range), mg/L	0.58 (0.01-2.59)
hsCRP, n (%)	
Normal (<1 mg/L)	70 (87.5)
High (≥1 mg/L)	10 (12.5)
Mean leptin (SD), ng/dL	20.68 (11.03)
Median adiponectin (range), pg/dL	5.25 (2.5-35)
Median TNF-α (range), µg/dL	3,106 (1,737-8,399)

Table 3. Correlations between hsCRP with body fat mass, leptin, adiponectin, and TNF-

Variables	hsCRP level	
	r	P value
Leptin	0.185	0.101
Adiponectin	-0.095	0.401
TNF-α	0.117	0.302
Body fat mass	0.383	<0.0001

body fat mass is shown in Figure 1. The hsCRP level increased along with body fat mass, reaching its peak at body fat mass of 28 kg. Beyond this point, hsCRP level tended to be stable (**Figure 1**).

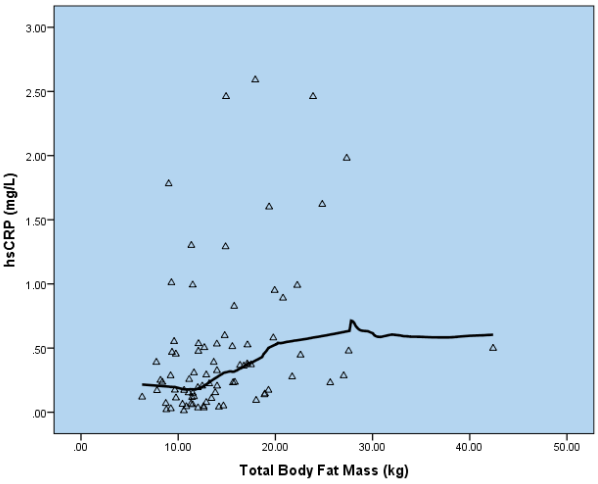

Figure 1. Scatter plot between body fat mass and

Discussion

In our study, 12.5% subjects had low-grade chronic inflammation, as defined by high hsCRP level. Low-grade chronic inflammation generates oxidative stress that exacerbates adipocyte malfunction in maintaining metabolic homeostasis. Energy excess is stored in adipocytes, resulting in hypertrophy and hyperplasia of adipocytes.[12,13] Many studies in the past two decades found that high hsCRP level was significantly associated with cardiovascular and metabolic diseases, including hypertension, coronary arterial disease, peripheral arterial thrombosis, stroke, and metabolic syndrome.[14-16] It is still unclear whether hsCRP plays a causative role in the pathogenesis or is only a marker of inflammation. However, several studies found that hsCRP has important roles in opsonization, uptake of lipid by macrophages, increased recruitment of monocytes into arterial plaque, dysregulation of endothelial function by disrupting nitric oxide release, and increased expression of plasminogen activator inhibitor-1 (PAI-1) on endothelial surfaces.[14,17]

Leptin, especially in its free form, plays a major role in regulation of energy balance. Although the concentration of leptin in an obese person is higher than in a normal weight person, it loses its function of controlling food intake. This condition is defined as leptin resistance.[6,18] We did not find a correlation between hsCRP and leptin levels. It can be explained by the fact that leptin is involved in innate and adaptive immune system maturation, activation, and proliferation.[18,19] The lack of significance was possibly because many factors, including dietary intake, environmental temperature, and stress stimulus, can affect leptin production.[21-23]

There was an inverse trend between hsCRP and adiponectin levels, but it was also not significant. The inverse trend is possibly because adiponectin has anti-inflammatory properties by reducing inflammatory cytokines (TNF-α, nuclear factor-κB, vascular cell adhesion molecule-1, E-selectin, and IL-8), inhibiting transformation of macrophage to foam cells, and enhancing production of nitric oxide (NO). Adiponectin plays a protective role in various obesity-related complications.[24] The lack of significance may have been due to genetic, environmental, and lifestyle factors, including sleep duration, which also influences serum adiponectin level.[25,26]

In this study, TNF-α was not correlated with hsCRP level. The TNF-α is produced by mature adipocytes and stromal-vascular cells, and neutralizes tyrosine kinase, causing insulin resistance, which may be aggravated by inflammation in obesity.[27,28] Several studies found that hsCRP and TNF-α levels

concurrently increased during various metabolic diseases.[29-31] However, none of these studies analyzed for correlations between the markers. One study of type 2 diabetes mellitus patients found that hsCRP was not related with the basal value of TNF-α.[32]

We found a positive, weak correlation between hsCRP level and body fat mass. Adipocytes play an important role in causing chronic states of inflammation in obese people. Adipocyte dysfunction in obesity enhances production of reactive oxygen species and pro-inflammatory cytokines.[33] The positive peak of hsCRP level was reached at a body fat mass of 28 kg. This observation may imply that adipocytes have limitation in producing pro-inflammatory substances in response to metabolic failure.

This study has several limitations. A further study with a larger sample size, controlled diet, and normal weight boys as a control group are needed to evaluate the relationship between the markers mentioned above.

In conclusion, low-grade, chronic inflammatory state (high hsCRP level) in obese children begins at a young age. The level of hsCRP has a weak correlation with body fat mass, but no correlation with adipocytokines. Childhood obesity should be prevented or treated as early as possible to prevent further cardiovascular and metabolic diseases.

Conflict of interest

None declared.

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Case Report

Baby girl with pentalogy of Cantrell: a case report on an extremely rare condition

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In 1958, Cantrell *et al.* described an extremely rare syndrome of congenital defects involving the abdominal wall, sternum, diaphragm, pericardium, and heart malformation.[1] The incidence of pentalogy of Cantrell (POC) is one in 65,000 live births.[1,2,3] Only one case was reported in Dr. Moewardi Public Hospital between 1999 and 2016. The exact etiology of this condition is unknown, but developmental failure of mesoderm at 4 weeks of gestational age may contribute to the condition.[4] The prognosis depends on the degree of heart failure and the malformations that occur. Heart failure, arrhythmia, cardiac rupture, cardiac tamponade, endocarditis, and peripheral emboli are described as the main complications and causes of death.[5,6] The aim of this report was to add to reference data about complete POC and the prognostic outcome. **[Paediatr Indones. 2019;59:51-4; doi: http://dx.doi.org/10.14238/pi59.1.2019.51-4].**

Keywords: *pentalogy of Cantrell; ectopia cordis; omphalocele*

The Case

A baby girl weighing 2,800 grams was born full term, cried spontaneously and had no cyanosis. Her mother was 20 year old and this was her first pregnancy. The baby was admitted to our hospital due to a big lump on her abdominal wall. There was no family history of congenital defects. At birth, the baby's vital signs were normal and the ictus cordis (the apex beat) appeared normal with regular heart sounds. Physical examination showed a palpable soft mass resembling omphalocele (4x5x6cm) with ectopia cordis on the supraumbilical defect (**Figure 1**). Electrocardiography demonstrated sinus rhythm with left ventricular hypertrophy and echocardiogram showed defects of the subaortic ventricular septum, left-to-right shunt, and moderate pulmonal stenosis with 55% ejection fraction (**Figure 2**).

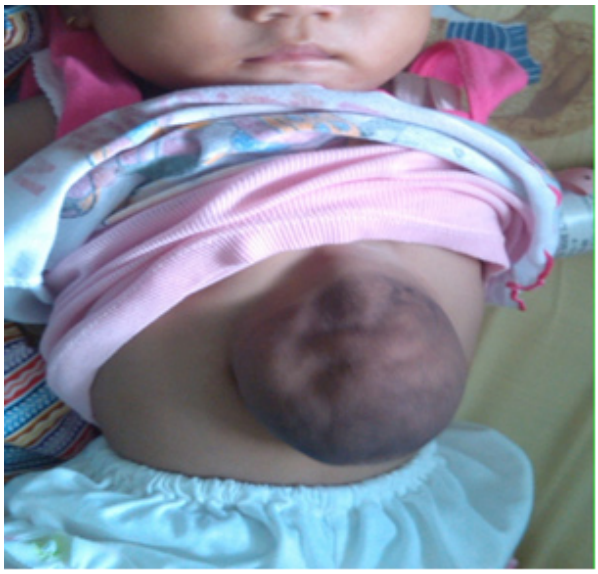

Figure 1. A palpable soft mass resembles omphalocele (4x5x6cm) with ectopia cordis on the s praumbilical defect.

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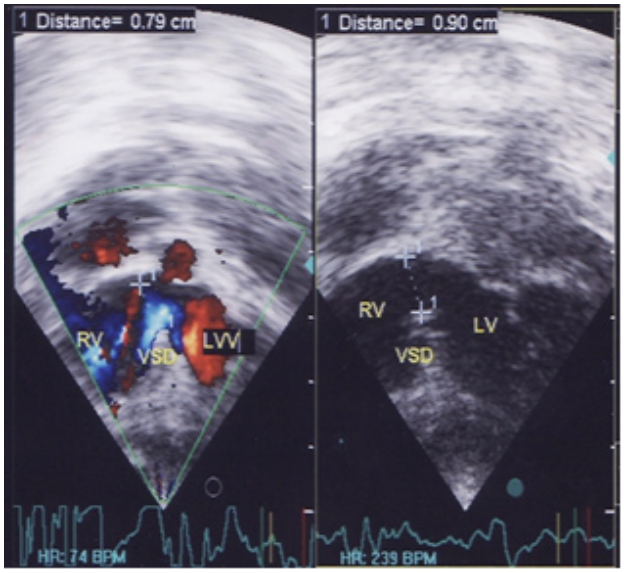
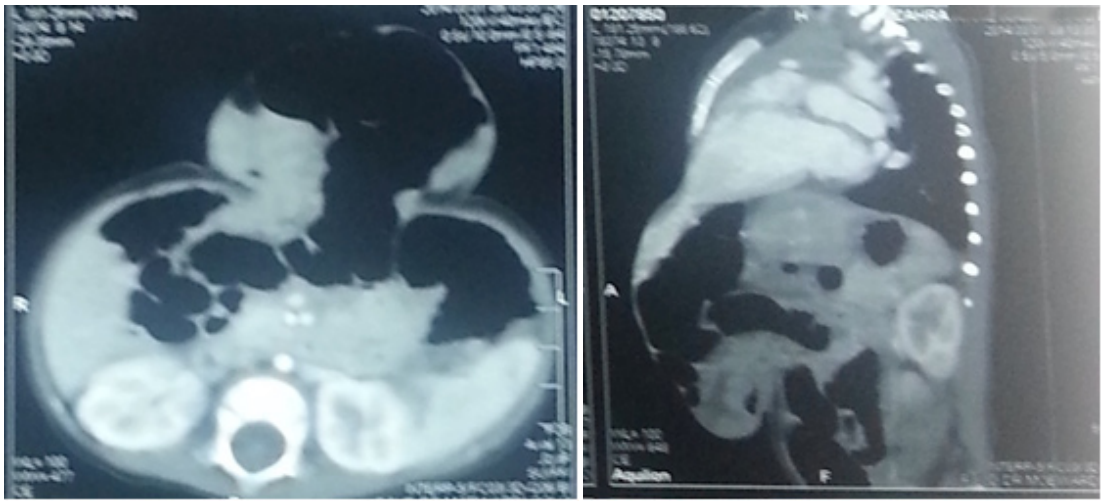


Figure 2. Electrocardiography demonstrated sinus rhythm with left ventricular hypertrophy, while echocardiography showed subaortic ventricular septum defect, left-to-right shunt, and moderate pulmonal stenosis with ejection fraction 55%.

The thoraco-abdominal CT scan with contrast was performed due to the cardiac abnormalities cited above and it revealed a huge cardiac diverticulum starting from the left ventricular apex to the umbilicus, accompanied by omphalocele. Supraumbilical defects followed by a defect in the lower sternum of 62.1 mm in diameter (**Figures 3a and 3b**).

The left ventriculography catheterization revealed adequate right ventricle size and a flow from the right ventricle to the pulmonary artery. Right ventriculography revealed confluent pulmonary artery, visible left diaphragmatic hernia with intestine in front of the left lung, and heart protruding forward. The left ventricle had a visible ventricular septal



Figures 3 (a. axial view, b. sagital view). The contrasted thoracoabdominal CT scan showed asserting cardiac abnormalities and a huge cardiac diverticulum starting from the left ventricular apex to the umbilicus, accompanied by omphalocele as well as supraumbilical defects with the defect in the lower sternum of 62.1 mm in diameter.

defect with left-to-right shunt. Aortography showed no collateralization and no patent ductus arteriosus. During the catheterization, the patient had ventricular tachycardia and died in the pediatric intensive care unit due to heart failure.

Discussion

Cantrell et al. first described the full spectrum of POC in 1958.[1] Cantrell's pentad, it is an extremely rare congenital malformation and usually lethal, with multiple defects in the midline supraumbilical abdominal wall, anterior diaphragm, sternum, as well as diaphragmatic, pericardial, and heart defects.[1-5] Complete POC is rarely reported. Thus, we report a case of complete POC, with hopes of improving our knowledge on this extremely rare congenital condition.

Toyama described the following classifications of POC: Class 1- complete syndrome with all five defects present; Class 2- probable syndrome, with four defects present, including intracardiac and ventral abdominal wall abnormalities; and Class 3- incomplete syndrome with various combinations of defects present, always with sternal abnormalities.[6] The failure of pathophysiology and embryologic development during pregnancy results in various abnormalities, especially those derived from the mesoderm layer.1-5 The extracardiac disorders include omphalocele, anterior diaphragmatic defects, sternal defect, may also be observed in association with ectopia cordis.[7]

Omphalocele should be considered pathologic only, if it persists beyond 14 weeks or if its maximum diameter exceeds 1 cm in the first trimester of pregnancy.[8] The sternal and abdominal wall defects represent faulty migration of these mesodermal primordial structures. It is thought that these developmental abnormalities occur from approximately days 14 to 18 of embryonic life.[8] The anterior abdominal wall defects are mostly omphalocele. A retrograde ventral diaphragm defect occurs in 91% of cases. Mortality is higher in infants with the complete form of POC and associated with extracardiac anomalies.[9] In the literature, thoraco-abdominal ectopia cordis (EC) is the usual type of ectopia cordis associated with Cantrell's pentalogy. Ectopia cordis is defined as an abnormal location of the heart, partially or totally outside of the thorax. Both are in the cervical, cervicothoracic, chest, and thoracoabdominal regions.[10] The incidence of ectopia cordis is 5.5-7.9 per one million births, and only about 0.5 to 0.8% of all congenital heart diseases.[7] Ectopia cordis is mostly associated with other congenital heart anomalies, including ventricular septal defect, atrial septal defect, pulmonary stenosis, Tetralogy of Fallot, and right ventricular diverticulum.[7,10] Mortality in children with EC is high; no more than 5% survive.[9] The POC usually can be diagnosed in the first trimester of pregnancy with prenatal ultrasonography, usually made by 2-dimensional ultrasound examination, whereas 3-dimensional ultrasonography is more useful in making a diagnosis in the second and third trimesters.[10,11]

In our case, the sternal defects were the absence of xiphoideus and the lower 2/3 of the sternum. Cardiac rupture, tamponade, sudden death, endocarditis, peripheral embolism, heart failure, and arrhythmia have all been described as complications and causes of death. Sternal defects include bifid sternum (26%), absent xiphoid (10%), and absent lower 2/3 of sternum (9%). A ventral retrosternal defect of the diaphragm occurs in 91% of the cases.[11]

In conclusion, the pathogenesis of POC is uncertain. It arises from an embryological development defect occurring in a segment of lateral mesoderm, 14-18 days after conception. The survival rate for patients with complete POC is low, with the prognosis depending on the severity of the cardiac defect and complexity of the malformations.

Conflict of Interest

None declared.

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Original Article

Risk factors for delayed speech in children aged 1-2 years

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Abstract

Background Speech delay is one of the most common developmental delays in children. To minimize the negative outcomes of speech delay, risk factors should be explored to help in early patient diagnosis.

Objectives To assess for associations between delayed speech in children aged 1 to 2 years and possible risk factors including gender, gestational age, birth weight, asphyxia during birth, head circumference, anterior fontanelle closure, gross motor development, duration of breastfeeding, caregiver identity, number of siblings, exposure to gadgets and television, and social interaction.

Methods Parents of children aged 1 to 2 years who were treated at Dr. Cipto Mangunkusumo Hospital, and Klinik Anakku, Pondok Pinang in Jakarta from January 2018 to March 2018 were interviewed. Data were processed with SPSS Statistics for Mac and analyzed by Chi-square test and logistic regression method.

Results Of 126 subjects, 63 children had speech delay and 63 children had normal speech development. Multivariate analysis revealed that the significant risk factors for delayed speech were delayed gross motor development (OR 9.607; 95%CI 3.403 to 27.122; P<0.001), exclusive breastfeeding for less than 6 months (OR 3.278; 95%CI 1.244 to 8.637; P=0.016), and exposure to gadgets and television for more than 2 hours daily (OR 8.286; 95%CI 2.555 to 26.871; P<0.001).

Conclusion Delayed gross motor development, exclusive breastfeeding for less than 6 months, media exposure for more than 2 hours daily, and poor social interaction are risk factors for delayed speech development in children. **[Paediatr Indones. 2019;59:55-62; doi: http://dx.doi.org/10.14238/pi59.2.2019.55-62]**.

Keywords: *breastfeeding; gadget; risk factors; speech delay*

Speech delay is one of the most common developmental delays in children, with a reported prevalence of 5-8% amongst children aged 2 to 4.5 years in 2006.[1] This percentage was lower compared to two decades before, when it was 3-10%.[2] In Indonesia, Dr. Kariadi Hospital in Semarang in 2007 encountered 100 children with speech delay out of the 436 children tested.[3] Data obtained by Dr. Cipto Mangunkusumo Hospital showed that 10.13% from 1125 children visits in 2006 were tested positive for speech delay.[3] More studies should be carried out to obtain a timely prevalence in both Indonesia and worldwide.

Normal speech progresses through stages, starting with cooing at the age of 3 months, continuing with babbling, imitation of sounds, jargon, and single words, word combinations, and finally sentence formation.[4] Some children may progress at a slower pace compared

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to other children, hence the term, speech delay.[5] In such cases, parents or caregivers should pay close attention to speech development of their children, as there are red flags that suggest the need for immediate intervention by physicians. If not managed properly, speech delay may impact a person's life personally, socially, academically, and vocationally for years to come.[5]

Early detection of speech delay is crucial, in order to intervene as early as possible.[6] Early intervention can help these children have a longer time window to catch up in their development.[7] As such, we determined to identify risk factors of speech delay in children. The factors assessed were gender, gestational age, birth weight, asphyxia during birth, head circumference, closure of anterior fontanelle, gross motor development, duration of breastfeeding, caregiver identity, number of siblings, exposure to gadgets and television, and subject's social interaction. Subjects with ages ranging from 1 to 2 years were chosen because this is the period in which brain growth is most rapid during the first 1,000 days of life. The lower age limit of 1 year was chosen because this is normally the age when a child starts to say 1 to 2 meaningful words.[4] The upper age limit of 2 years old was chosen so that detection can be done to allow for at least one year of intervention, in order to give the children a chance to meet normal speech development by the end of the first 1,000 days.

Methods

This study was conducted from August 2017 to April 2018 in Dr. Cipto Mangunkusumo Hospital and Klinik Anakku, Pondok Pinang in Jakarta, Indonesia. Our matched, case-control study included 126 children aged 1 to 2 years. Subjects were classified into two groups: the control and case group, based on fulfillment of the control/case inclusion criteria. Subjects with normal speech development, as indicated by their ability to fulfill the milestones of normal expressive speech and language development milestones (**Table 1**), were placed in the control group. Subjects in the case group had delayed speech development. Children with apparent syndromes or abnormalities in or around the mouth were excluded from this study. This study was approved by the Ethics Committee of the Universitas Indonesia Medical School.

Data collection was done with a purposive sampling method. Subjects' parents or guardians were interviewed using a questionnaire to obtain information about the subjects' gender, gestational age, birth weight, asphyxia during birth, head circumference, closure of anterior fontanelle, gross motor development, duration of breastfeeding, caregiver identity, number of siblings, exposure to gadgets and television, and social interaction of subject. The questions regarding these variables were available in the questionnaire. Head circumference, closure of anterior fontanelle, and subject's social interaction were measured directly on the subjects.

Data were processed by *SPSS Statistics for Mac* and analyzed by Chi-square test and logistic regression method. The significance level in both tests was $P < 0.05$.

In this study, speech delay was defined as a slower progression of speech compared to other children of the same age. Subjects born at under 35 weeks were considered to be premature, and those with birth weight <2,500 grams were considered to have low birth weight. Subjects who did not cry right after delivery were considered to have experienced asphyxia during birth. Subjects who scored below -2 SD (2%) in the Nellhaus head circumference chart were considered to have microcephaly.[8] Subjects' anterior fontanelle sizes were measured and compared to mean anterior fontanelle size in **Table 2** to assess closure.

Table 1. Milestones for expressive speech and language in children[7]

Age (months)	Expressive skills
0-2	Cries
3	Coos Laughs
6	Babbles
9	Imitates sound
12	1-2 meaningful words
18	At least 6 meaningful words
24	Forms 2-3-word sentences

Table 2. Mean of anterior fontanelle size[16]

Age, months	Mean size (SD), cm
9-12	1.15 (1.2)
12-18	0.05 (0.22)
18-24	0 (0)

Subjects who did not fulfill the following milestones were considered to have delayed gross motor development: 12 months - walks with 1 hand held, 15 months - walks alone, 16 months - runs, 18 months - walks upstairs with assistance, and 24 months - jumps. Short duration of breastfeeding was defined as < 6 months of exclusive breastfeeding. Caregiver identity was defined as mother or babysitter/ others. Children were considered to have minimal attachment to the mother if they were nurtured by a babysitter or a family member other than the mother. A subject was considered to have a sibling if he was not the only child in the family (either related or unrelated by blood). Subjects who spent more than 2 hours/ day exposed to media (gadgets and/or television) were considered to have excessive media exposure. Direct examination was done to measure head circumference, closure of anterior fontanelle (and size of anterior fontanelle if not closed), and the subject's social interaction. Subjects who responded poorly or did not respond at all during social interaction were considered to have poor social interaction.

Results

Of 126 subjects, 75 (59.53%) were male; 63 subjects had speech delay and the other 63 had normal speech development. All subjects were aged 1 to 2 years. Subjects' characteristics are shown in **Table 3**.

Of 126 subjects in our study, 34 children (27.0%) were aged 12 to 15 months, 28 children (22.2%) aged 16 to 18 months, 30 children (23.8%) aged 19 to 21 months, and 34 children (27.0%) aged 22 to 24 months. Most subjects with speech delay were in the 22-24 month age group, with 14 children, accounting for 22.2% of the population with speech delay.

Bivariate analysis (**Table 3**) showed that out of the 12 variables analyzed, 5 were significantly related to speech delay: asphyxia during birth (OR 3.625; 95%CI 1.229 to 10.695; P=0.028), gross motor development not according to milestones (OR 9.750; 95%CI 4.086 to 23.267; P<0.001), duration of exclusive breastfeeding for <6 months (OR 3.558; 95%CI 1.694 to 7.471; P=0.001), exposure to gadgets and television for >2 hours daily (OR 7.125; 95%CI 2.679 to 18.948; P<0.001), and poor social interaction (OR 0.432; 95%CI 0.349 to 0.535; P<0.001). The remaining seven variables had P values >0.05, thus were not significant.

The full multivariate logistic regression model (**Table 4**) included the nine variables with P values < 0.25 in bivariate analysis. In the final model (**Table 5**), three variables were found to be significant, namely, gross motor development (OR 9.607; 95%CI 3.403 to 27.122; P<0.001), duration of exclusive breastfeeding (OR 3.278; 95%CI 1.244 to 8.637; P=0.016), and exposure to gadgets and television (OR 8.286; 95%CI 2.555 to 26.871; P < 0.001).

Discussion

There was a participation of 126 children in this study, in which 50% of the population had a delayed speech development. Mondal *et al.*[10] in 2016 assessed 200 children aged 0 to 36 months and found a prevalence of children aged 13 to 24 months with speech delay was 14.5%, which was lower than the results of this study (50%).

Gender was not significantly associated with speech delay (OR 2.083; 95%CI 1.009 to 4.300; P=0.07), despite more males (68.3%) than females (31.7%) found to have delayed speech development. In contrast, Keegstra et al. in 2006 and Mondal et al. in 2016 found that significantly more males than females had speech delay.[10, 11]

Mondal *et al.* also noted that gestational age and birth weight were not significantly associated with speech delay [(OR 0.4; 95%CI 0.52 to 3.74; P=0.67) and (OR 1.3; 95%CI 0.56 to 2.91; P=0.296), respectively].[10] Similarly, we also found no significant associations between speech delay and gestation age (OR 2.286; 95%CI 0.854 to 6.121; P=0.151), or birth weight (OR 1.700; 95%CI 0.739 to 3.911; P=0.296). The only perinatal factor assessed in our study was perinatal asphyxia, and this factor was found to be significant with regards to speech delay (OR 3.625; 95%CI 1.229 to 10.695; P=0.028). In addition, Nguefack *et al.* in 2013 stated that perinatal asphyxia was the most frequent perinatal factor to cause developmental delay (44%; P=0.05).[12]

Perinatal hypoxia-ischemia is responsible for primary and secondary cerebral energy failure, a phenomena in which the blood flow to the brain is decreased, thus reducing oxygen transport. These

Table 3. Characteristics of subjects (N=126)

Characteristics	Normal (n=63)	Speech delay (n=63)	OR (95%CI)	P value
Gender, n(%)				
Female	31 (49.2)	20 (31.7)	2.083	0.070
Male	32 (50.8)	43 (68.3)	(1.009 to 4.300)	
Gestational age, n (%)				
≥ 35 weeks	56 (88.9)	49 (77.8)	2.286	0.151
< 35 weeks	7 (11.1)	14 (22.2)	(0.854 to 6.121)	
Birth weight, n (%)				
≥ 2,500 grams	51 (81.0)	45 (71.4)	1.700	0.296
< 2,500 grams	12 (19.0)	18 (28.6)	(0.739 to 3.911)	
Asphyxia during birth, n (%)				
No	58 (92.1)	48 (76.2)	3.625	0.028*
Yes	5 (7.9)	15 (23.8)	(1.229 to 10.695)	
Head circumference, n (%)				
Normal	51 (81.0)	44 (69.8)	1.835	0.215
Microcephaly	12 (19.0)	19 (30.2)	(0.802 to 4.199)	
Closure of anterior fontanelle, n (%)				
Yes	44 (69.8)	48 (76.2)	0.724	0.547
No	19 (30.2)	15 (23.8)	(0.328 to 1.596)	
Gross motor development, n (%)				
According to milestones	54 (85.7)	24 (38.1)	9.750	< 0.001*
Not according to milestones	9 (14.3)	39 (61.9)	(4.086 to 23.267)	
Duration of exclusive breastfeeding, n (%)				
> 6 months	45 (71.4)	26 (41.3)	3.558	0.001*
< 6 months	18 (28.6)	37 (58.7)	(1.694 to 7.471)	
Caregiver, n (%)				
Mother	51 (81.0)	43 (68.3)	1.977	0.152
Babysitter (or not mother)	12 (19.0)	20 (31.7)	(0.868 to 4.500)	
Number of siblings, n (%)				
≥ 1	13 (20.6)	17 (27.0)	1.421	0.530
0	50 (79.4)	46 (73.0)	0.622 to 3.246	
Exposure to gadgets and television, n (%)				
≤ 2 hours/day	57 (90.5)	36 (57.1)	7.125	< 0.001*
> 2 hours/day	6 (9.5)	27 (42.9)	(2.679 to 18.948)	
Social interaction, n (%)				
Good	63 (100.0)	48 (76.2)	0.432	< 0.001*
Poor	0 (0.0)	15 (23.8)	(0.349 to 0.535)	

*significant P value < 0.05

Table 4. Results of multivariate logistic regression analysis (full model)

Variables	B	OR (95%CI)	P value
Male gender	0.329	1.390 (0.505 to 3.827)	0.524
Gestational age (< 35 weeks)	0.898	2.454 (0.633 to 9.512)	0.194
Asphyxia during birth (yes)	0.751	2.119 (0.409 to 9.164)	0.751
Head circumference (microcephaly)	-0.354	0.702 (0.184 to 2.673)	0.604
Gross motor development (not according to milestones)	2.371	10.705 (3.124 to 36.687)	< 0.001*
Duration of exclusive breastfeeding (< 6 months)	1.122	3.071 (1.121 to 8.417)	0.029*
Caregiver (babysitter/or not mother)	0.436	1.546 (0.407 to 5.090)	0.473
Exposure to gadgets and television (> 2 hours/day)	2.123	8.354 (2.486 to 28.071)	0.001*
Social interaction (poor)	19.991	48,076.39 (< 0.001)	0.998
Constant	-2.515	0.081	< 0.001

*significant P value < 0.05

Table 5. Results of multivariate logistic regression analysis (final model)

Variables	B	OR (95%CI)	P value
Gross motor development (not according to milestones)	2.262	9.607 (3.403 to 27.122)	< 0.001*
Duration of exclusive breastfeeding (< 6 months)	1.187	3.278 (1.244 to 8.637)	0.016*
Exposure to gadgets and television (> 2 hours/day)	2.115	8.286 (2.555 to 26.871)	< 0.001*
Social interaction (poor)	20.218	60,305.42(< 0.001)	0.998
Constant	-2.046	0.129	< 0.001

*significant P value < 0.05

phenomena are also responsible for decreased high-energy phosphorylated compounds. The decrease in blood flow, oxygen transport, and high-energy phosphorylated compounds may lead to brain injury, resulting in immediate neuronal death (in primary cerebral energy failure), and delayed neuronal death (in secondary cerebral energy failure). Both neuronal deaths have adverse effects on neurodevelopment, including delayed speech development.[13]

Neither head circumference nor closure of anterior fontanelle was significantly associated with speech delay in our study [(OR 1.835; 95%CI 0.802 to 4.199; P=0.215) and (OR 0.724; 95%CI 0.328 to 1.596; P=0.547), respectively]. However, Davidovitch *et al.* found a significant relationship between head circumference and speech delay (P=0.03).[14] To our knowledge, no previous study has investigated the relationship between delayed closure of anterior fontanelle and speech delay. According to Esmaeili et al., delayed closure of the anterior fontanelle results from stunted brain growth, often manifesting as motor and speech delay, as well as cognitive impairment.[9]

Gross motor development was another developmental delay significantly associated with speech delay. Children with delayed gross motor development were more likely to experience delayed speech development (OR 9.750; 95%CI 4.086 to 23.267; P<0.001). In our study, of 63 children with speech delay, 61.9% had delayed gross motor development. A previous study compared infants who were dependent walkers (infants in baby-walker) with independent walkers, in terms of their vocalizations and social interaction. The latter group of walkers scored better in both variables tested.[15] Another previous study found that in children who accomplished milestones of standing with assistance by 2.1 months later scored 21.9 points less than children with normal gross motor milestones in the *Batelle Developmental Inventory*, 2nd edition (BCI-2) (95%CI -41.5 to -2.2).[16] These studies suggest that gross motor development is an important basis for speech development. Motor development may enhance language development in children by providing more opportunities to experience the world.[17] Iverson argued that infants' rib cages are restricted before they are able to sit on their own. When infants are able to sit without assistance, their rib cage is freed, thus, they can breathe more efficiently and maintain subglottal pressure, which is essential in speech production.[17] Iverson added that as soon as infants start walking, they are able to bring objects of interest to adults around them. By focusing on the object in their hands, infants are more likely to learn words related to that object. One of the many ways to increase an infant's interest in the objects around them is to entertain them by engaging their interest.[17] In other words, motor development such as a change in posture and locomotion, supported by object-manipulation, is highly stimulating to an infant's speech development later in life.[17]

The beneficial relationship of breastfeeding to both growth and development of children has been studied for years. We, too, noted that children with a breastfeeding duration of less than the recommended 6 months were at risk of developing speech delay (OR 3.5568; 95%CI 1.694 to 7.471; P=0.001). This result was comparable to that of a longitudinal study by Vestergaard *et al.*, which stated that children with increased duration of exclusive breastfeeding would display early speech and language skills that are indicated by polysyllable babble at the age of 8 months old.[18] The presence of polysaturated fatty acid such as omega-3 [docosahexaenoic acid (DHA)] and omega-6 [arachidonic acid (AA)] in breast milk has been suggested as the mechanism underlying this relationship. Both of these fatty acids are responsible for promoting neural growth and the development of white and gray matter, hence, exclusive breastfeeding for 6 months is highly correlated with higher language and cognitive scores.[19,20]

Similar to a study by Suparmiati *et al.* in 2013, no significant relationship was observed between caregiver (mother vs. babysitter or other) and speech delay (OR 1.977; 95%CI 0.868 to 4.500; P=0.152). However, care given by a babysitter may increase the risk or worsen delayed speech development in children, as shown in a previous study.[21]

There was also no significant relationship between the number of siblings and speech delay (OR 1.421; 95%CI 0.622 to 3.246; P=0.530) in our study. In comparison, Keegstra *et al.* found that an only child in the family had a higher chance of developing speech delay than a child with brothers and sisters (P=0.023).[11]

Exposure to gadgets and television for >2 hours daily was significantly associated with speech delay (OR 7.125; 95%CI 2.679 to 18.948; P<0.001). Out of the 63 children with speech delay, 42.9% were exposed to media for >2 hours daily. Only 9.5% of children without speech delay had media exposure of >2 hours daily. This result was consistent with a study by Duch *et al.* who found that exposure to gadgets and television of >2 hours daily was significantly associated with lower communication scores.[22] Hypotheses on the mechanism of how media may affect speech development have been proposed. Evidence suggests that young children are not proficient in learning words from media. Thus, exposing them to gadgets and television worsens their language acquisition by decreasing the quantity and quality time of the parent-child relationship and children's play activities.[22]

The last variable assessed in our study was social interaction, which was significantly related to speech development in children (OR 0.432; 95%CI 0.349 to 0.535; P<0.001). All children with normal speech development had good social interaction. In comparison, of the 63 children with delayed speech, 48 displayed good social interaction (76.2%) while the remaining 15 children (23.8%) displayed poor social interaction. To our knowledge, no previous study has hypothesized the relationship between the two variables, but Rice *et al.* found that children with delayed speech development have worse social interaction than children with normal speech and language development, which supported the finding in this study.[23]

Delayed gross motor development, <6 months of exclusive breastfeeding, and >2 hours of media exposure daily were significantly related to speech delay in the final multivariate logistic regression model [(OR 9.607; 95%CI 3.403 to 27.122; P<0.001), (OR 3.278; 95%CI 1.244 to 8.637; P=0.016), and (OR 8.286; 95%CI 2.555 to 26.871; P< 0.001), respectively]. A study by Chonchaiya *et al.* also showed a significant association between excessive media exposure and speech delay, with an odds ratio of 5.70 (95%CI 1.85 to 17.61).[24] In addition, Yanuarti et al. in Bandung, Indonesia, showed a relationship between non-exclusive breastfeeding and speech delay (PR 174.756; 95%CI 10.407 to 2,935.516; P<0.001).[25] Furthermore, the relationship between delayed gross motor development and delayed speech were significant in our study, but not in the study by Ghassabian *et al.*[16]

Social interaction was not significant in the final model for multivariate logistic regression (P=0.998). Further investigations are needed to confirm the relationship between social interaction and speech delay.

The limitation of this study was that parents may have provided inaccurate and incomplete information, especially on their child's gross motor development. For subsequent studies, it is recommended for researchers to conduct direct observation of gross motor development for all subjects. This study shall benefit clinicians, especially pediatricians, in educating parents of children with suspected speech delay. Verbal counselling is one of the preferred ways to educate parents on the practical changes to be made in their home environment to reduce the risk of speech delay, such as limiting their children's screen time.

In conclusion, delayed gross motor development, duration of exclusive breastfeeding of less than 6 months, and media exposure for more than 2 hours daily, are the significant risk factors of delayed speech development in children.

Conflict of Interest

None declared.

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Original Article

Prognostic factors of heart failure in children with left-to-right shunt acyanotic congenital heart disease

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Abstract

Background Anemia is highly prevalent and affects morbidity and mortality in adults with acquired heart disease. However, its role in children with acyanotic congenital heart disease (CHD) is unclear.

Objective To assess anemia and other potential prognostic factors of congestive failure in children with left-to-right shunt acyanotic CHD.

Methods We conducted a case-control study in the Pediatric Cardiology Clinic, Dr. Sardjito Hospital from January to December 2017 in children with left-to-right shunt acyanotic CHD. The case and control groups consisted of subjects with and without heart failure, respectively. Anemia was defined as hemoglobin concentration <11 g/dL. Measured outcome was the prevalence of congestive heart failure, as determined by the Ross criteria. Anemia, defect type, defect size, age at diagnosis, and gender were analyzed by logistic regression analysis as potential predictive factors of heart failure.

Results Of 100 children with left-to-right shunt acyanotic CHD, 50 had heart failure (the case group) and 50 did not (the control group). The prevalence of anemia was 45%. Multivariable logistic regression revealed that defect size was the most significant factor for predicting heart failure, with adjusted OR 7.6 (95%CI 2.5 to 22.8) for moderate shunts and 21.1 (95%CI 6.8 to 65.4) for large shunts. Anemia, type of defect, age of diagnosis, and gender were not statistically significant factors for predicting outcomes.

Conclusion Anemia is not a significant, prognostic factor for heart failure in children with left-to-right shunt acyanotic CHD. However, moderate and large shunts in children with left-to-right shunt acyanotic CHD are predictive of the occurrence of congestive heart failure. **[Paediatr Indones. 2019;59:63-6; doi: http://dx.doi.org/10.14238/pi59.2.2019.63-6]**.

Keywords: *anemia; heart failure; congenital heart disease; prognostic factors*

Anemia is a common nutritional problem that is common in both developed and developing countries. The prevalence of anemia in children in developing countries is around 39%.[1] Anemia increases cardiovascular disease morbidity and mortality caused by compensatory mechanisms of hypoxia, that is by increased heart rate, increased cardiac output, left ventricular hypertrophy and progressive heart enlargement, as well as reduced oxygenation to the heart muscle.[2] Congestive heart failure can occur in patients with congenital heart abnormalities such as atrial septal defect, ventricular septal defect, patent ductus arteriosus, and other structural heart abnormalities that cause increased pulmonary blood circulation volume. Anemia increases the burden on the heart to fulfill tissue oxygen demand, which aggravates heart failure. Anemia increases the burden of heart to fulfill tissue oxygen demand, so that will aggravate heart failure. Previous studies showed that anemia was a strong predictor of death in adult

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patients with acyanotic congenital heart disease (hazard ratio: 3.0; 95%CI 1.46 to 6.13).[2] Anemia increases the hospital length of stay due to heart failure in children with dilated cardiomyopathy [mean 35.1 (SD 40.5) *vs.* 9.97 (SD 9.65) days per year, respectively; P<0.05].[3] Hence, we aimed to assess the role of anemia and other prognostic factors of congestive heart failure in children with left-to-right shunt acyanotic CHD.

Methods

This was a case control study included children aged 1 month to 18 years with left to right shunt acyanotic CHD in the Pediatric Cardiology Clinic, Dr. Sardjito Hospital in 2017. The case group consisted patients with heart failure of class Ross 2 or more,[4] while the control group had patients without heart failure. Patients with chronic renal failure, malignancy, immunodeficiency, autoimmune disease, pulmonary stenosis, or multiple cardiac defects were excluded. The minimum required sample size for case-control study with beta strength 80% and alpha 0.05 significance was calculated to be 50 subjects for each group. Both case and control subjects were randomly selected by a computer program. Patient data obtained from medical records included the following possible prognostic factors of heart failure: anemia, age at diagnosis, gender, size of defect, and type of defect. Anemia was defined as hemoglobin level <11 g/dL. Chi-square test was performed to assess potential prognostic factors of heart failure. Odds ratio was calculated by a 2x2 table with 95% confidence intervals. Multivariable logistic regression analysis was performed on prognostic factors that had a Chi-square result of P<0.25, in order to determine adjusted odds ratios.

The study protocol had been approved by the Ethics Committee of the Universitas Gadjah Mada Medical School.

Results

Of the 145 patients who met the inclusion criteria, only 50 children were suitable for the case group, and 50 of 95 children were randomly selected for the control group. The proportions of heart defects in our subjects were 28% ASD, 36% VSD, and 36% PDA (**Table 1**). Large defects were seen in 35% of subjects, moderate defects in 33%, and small defects in 32%. Anemia prevalence was 45% of all subjects.

Table 1. Basic characteristics of subjects

Characteristics	(N = 100)
Male, n (%)	39 (39)
Median age (range), months	15.2 (1-204)
Mean heart rate (SD), x/minute	123 (20.3)
Median respiration rate (range), x/minute	32 (20-68)
Median Hb (range), g/dL	11.3 (7.4-20.2)
Defect type, n (%)	
ASD	28 (28)
VSD	36 (36)
PDA	36 (36)
Size of defect, n (%)	
Small	32 (32)
Moderate	32 (32)
Large	36 (36)

Hb=hemoglobin; ASD=atrial septal defect; VSD=ventricular septal defect; PDA=patent ductus arteriosus

Chi-square analysis revealed that anemia, age < 1 year at diagnosis, type of defect, and gender did not significantly differ between those with and without heart failure. Logistic regression analysis showed that subjects with moderate size defect had 7.6 times higher probability of congestive heart failure than small size defect. In addition, subjects with large size defect were 21.1 times more likely to have congestive heart failure than those with small size defects (**Table 2**).

Discussion

The prevalence of anemia was high in children with left-to-right shunt acyanotic congenital heart disease (45%). A previous study in Dr. M Djamil Padang Hospital reported a 34.4% prevalence of anemia in children with acyanotic congenital heart disease.[5] Although anemia was not a significant prognostic factor of congestive heart failure in children with acyanotic congenital heart disease, anemia remains a serious nutritional problem in this population. This prevalence of anemia in acyanotic congenital heart disease was higher compared to the prevalence of anemia in children aged 1-14 years in Indonesia of 27%.[6] Anemia was also associated with the risk of malnutrition (OR 6.5; 95%CI 4 to 8) in children with congenital heart disease.[7]

Table 2. Prognostic factors of congestive heart failure in children with left-to-right shunt acyanotic CHD

	CHF (n = 50)	No CHF (n = 50)	OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Anemia, n (%)	23 (46)	22 (44)	1.1 (0.5 to 2.4)	0.84		
Age of diagnosis > 1 year, n (%)	27 (54)	27 (54)	1 (0.5 to 2.1)	1		
Male, n (%)	16 (32)	23 (46)	1.8 (0.8 to 4.1)	0.15	1.8 (0.8 to 4.1)	0.18
Defect type, n (%)						
ASD	12 (24)	16 (32)				
VSD	18 (36)	18 (36)	1.3 (0.5 to 3.6)	0.57		
PDA	20 (40)	16 (32)	1.7 (0.6 to 4.5)	0.31		
Size of defect, n (%)						
Small	5 (10)	27 (54)				
Moderate	18 (36)	14 (28)	6.9 (2.1 to 22.7)	0.001	7.6 (2.5 to22.8)	0.001
Large	27 (54)	9 (18)	16.2 (4.8 to 54.7)	0.001	21.1 (6.8 to65.4)	0.001

Anemia is a predictor of death (OR 3; 95%CI 1.46 to 6.13) in adult CHD patients, and patients with anemia tend to have higher *New York Heart Association* (NYHA) classification of heart failure.[2] In patients with anemia, peripheral perfusion decreases as a result of a decreased vascular resistance. This condition may lead to neurohormonal activation with subsequent reduction in renal blood flow and renal function, resulting in an increase of intravascular volumes due to water and salt retention. The increased intravascular volume and decreased vascular resistance result in increased cardiac work, which may lead to left ventricular hypertrophy and worsening of cardiac function, as well as further decrease in renal function, completing the vicious circle.[8] The difference in the influence of anemia on acyanotic CHD in children and adults may be due to different characteristics of heart failure. Heart failure in CHD is due to impaired ventricular contractions in adults,[9] but related to pulmonary blood vessel congestion due to excessive blood flow through the left-to-right shunt in children.[10] This observation was consistent with our results showing that greater shunt size had higher risk of heart failure.

Regarding the high prevalence of anemia in children with acyanotic CHD, we recommend iron supplementation since the anemia may be caused by iron deficiency. Furthermore, children with moderate to large shunts should be prioritized for immediate defect closure. The limitation of this study was that subjects were only taken from one teaching hospital in Yogyakarta. As such, our results may not be generalized to other children with CHD in Indonesia or to other low-to-middle income settings.

Conflict of Interest

None declared.

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Original Article

Corticosteroids for pediatric septic shock patients

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Abstract

Background Septic shock remains a major cause of mortality and admission to the pediatric intensive care unit (PICU) in children. Management includes adequate fluid resuscitation, followed by catecholamine infusion, if needed. Corticosteroid therapy is advised for catecholamine-refractory shock, although this practice is controversial, as it was not beneficial in other studies.

Objective To assess corticosteroid use in pediatric septic shock patients in Cipto Mangunkusumo Hospital.

Methods This cross-sectional study included all patients aged 1 month-18 years with a diagnosis of septic shock during the study period of January 2014 to July 2018 admitted in PICU Dr. Cipto Mangunkuskumo Hospital, Jakarta. Data obtained from medical records were, age, sex, immunology status, port d'entrée of sepsis, inotropic and vasopressor usage, mechanical ventilation, corticosteroid type, hospital length of stay (LoS), and mortality outcome.

Results Of 217 children with septic shock, 12 patients (5.5%) received corticosteroid therapy. The most common corticosteroid given was hydrocortisone (80%), with a 2 mg/kg BW loading dose, followed by a continuous infusion dose of 2-50 mg/kg BW/day. Almost all patients (11/12) received corticosteroid therapy until they died. Median duration of corticosteroid use was 2 (range 1-7) days, median number of inotropes and vasopressors used was 3 (range 2-4) agents, median LoS was 3 (range 1-9) days, and mortality rate was 100%.

Conclusion A small proportion of pediatric septic shock patients received corticosteroid therapy. Their mortality rate was 100%. Further clinical study is needed to evaluate the benefit of corticosteroid therapy in pediatric septic shock patients. **[Paediatr Indones. 2019;59:67-71; doi: http://dx.doi.org/10.14238/pi59.2.2019.67-71]**.

Keywords: *corticosteroid; mortality; pediatric septic shock; septic shock*

Sepsis is defined as a life-threatening condition of organ dysfunction caused by a dysregulated host response to infection.[1] Sepsis is still one of the major causes of morbidity, and the leading cause of pediatric intensive care unit (PICU) admissions.[2] *The World Health Organization* (WHO) reported 80% mortality of children below 4 years of age with sepsis.[3] Based on medical record data in Cipto Mangunkusumo Hospital in 2009, the incidence of sepsis in children admitted to the PICU was 19.3% and the mortality rate was 10%. Also, 5-30% of pediatric sepsis cases can progress to septic shock.[4] Rusmawatiningtyas *et al.*[5] reported an 88.2% mortality rate in children with septic shock admitted to the PICU.

The American College of Critical Care Medicine (ACCM) developed an algorithm to target an initial resuscitation fluid of 20 mL/kg BW of crystalloid or colloid which can be repeated to 60 mL/kg BW, until perfusion improves or unless rales or hepatomegaly develop. For fluid refractory shock, vasopressors and inotropes must be given. If the shock does not resolve, it may progress to catecholamine-resistant shock.

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This condition is related to adrenal insufficiency, which requires corticosteroid administration such as hydrocortisone.

Several studies on intravenous hydrocortisone administration for catecholamine-resistant shock yielded varying results. Wong *et al.*[8] suggested that corticosteroid administration for catecholamine-resistant shock be reconsidered, due to side effects including gastrointestinal bleeding, delayed wound healing, hyperglycemia, and immunosuppression. In addition, Atkinson *et al.*[9] reported that corticosteroid administration in children with refractory septic shock had no benefits.

In Cipto Mangunkusumo Hospital, corticosteroid administration for pediatric septic shock has not been well studied. As such, we aimed to assess corticosteroid therapy for pediatric septic shock to see the prognosis or outcomes as a treatment consideration for future pediatric shock patients.

Methods

This cross-sectional study was done with patients' medical records in Cipto Mangunkusumo Hospital. Subjects were children aged 1 month to 18 years diagnosed with septic shock in the emergency room/PICU from January 2014 to July 2018. Patients with incomplete medical records were excluded from the study. Data obtained from medical records were, age, sex, immunology status, *port d'entrée* of sepsis, inotropic and vasopressor usage, and mechanical ventilation. The type, dose, and duration of corticosteroid therapy were collected for all subjects. Outcomes data were hospital LoS and mortality.

Results

From January 2014 to July 2018, 217 children were diagnosed with septic shock at Cipto Mangunkusumo Hospital, of whom 132 (60.8%) were male. Twelve patients received corticosteroid therapy for septic shock, 8 (67%) of whom were male. Subjects' median age was 165 (range 11-553) months. Six patients were immunocompromised, of whom 2 patients had systemic lupus erythematosus (SLE), 2 patients had poor nutritional status, 1 patient had lymphoma and poor nutritional status, and 1 patient received post-liver transplantation immunosuppressant drugs. The sources of sepsis were respiratory tract (6/12), abdominal organs (5/12), and urogenital tract (1/12). Patients' characteristics are described in **Table 1**.

Table 1. Characteristics of pediatric septic shock patients receiving corticosteroid therapy

Variables	(N=12)
Median age (range), months	165 (11-533)
Sex, n	
Male	8
Female	4
Immunity status, n	
Immunocompromised	6
Not immunocompromised	6
Port d' entrée sepsis, n	
Respiratory tract	6
Abdominal organ	5
Urogenital tract	1

The most common type of corticosteroid given was hydrocortisone (83%). One patient received dexamethasone as a hydrocortisone replacement. One remaining patient received hydrocortisone, followed by methylprednisolone as a hydrocortisone replacement. The hydrocortisone loading dose was 2 mg/kg (equal to 50 mg/m^2), followed by a continuous infusion dose of 2-50 mg/kg/day (equal to 50-1,250 mg/m^2/day). In our study, the maximum hydrocortisone dose recorded was 48 mg/kg/day (equal to 1,125 mg/m^2/day). The dexamethasone dose used was 1.8 mg/m^2/24 hours. One patient received hydrocortisone at a dose of 2 mg/kg/day (equal to 50 mg/m^2/day) that was increased gradually to 4 mg/kg/day (100 mg/m^2/day) in one day, before getting methylprednisolone at a dose of 0.4 mg/kg/day (equal to 10 mg/m^2/24 hours) that was increased gradually to 0.8 mg/kg/day (equal to 20 mg/m^2/day).

Corticosteroid doses were adjusted during administration. Most patients (58.3%) experienced that the corticosteroid dose was gradually increased until they died. However, one patient (8.3%) experienced tapering down to cessation of hydrocortisone during 7 days of administration. Almost all patients (92%) were still receiving corticosteroid therapy when they died. **Table 2** shows that the median duration of corticosteroid use was 2 (1-7) days. The median number of inotropes and vasopressors used was 3 (2-4). The

median LoS of patients with septic shock receiving corticosteroid therapy was 3 (1-9) days and the mortality rate was 100%.

Table 2. Outcomes of pediatric septic shock patients receiving corticosteroid therapy

Outcomes	(N=12)
Median duration of corticosteroid use (range), days	2 (1-7)
Median number of inotropic and vasopressor agents used (range)	3 (2-4)
Median LoS (range), days	3 (1-9)
Mechanical ventilation, n	12
Mortality, n	12

Discussion

The proportion of pediatric septic shock patients given corticosteroids was only 12 of 217 patients (5.5%) in the 4-year period at Cipto Mangunkusumo Hospital. Corticosteroid therapy was given when shock had not resolved after administering a minimum two types of vasopressors and inotropes. In contrast, Chrysostomos *et al*.[10] noted a larger proportion of septic shock patients receiving corticosteroids because the corticosteroid administration did not depend on the number, but on the duration, of inotropes and vasopressors used. They found that early initiation of corticosteroid administration, after 9 hours vasopressor and inotrope administration, resulted in better prognoses compared to late initiation of corticosteroid administration in patients with catecholamine-resistant septic shock.[10]

We found high usage of inotropic and vasopressor agents prior to corticosteroid therapy in our subjects, with median of 3 (range 2-4) drugs. Although corticosteroid therapy was usually given to patients after a combination of a minimum of two inotropic and vasopressor agents with maximum dose did not overcome shock, there was no standard protocol among physicians for starting corticosteroid therapy in such cases. In comparison, Menon *et al*.[11] reported that corticosteroid therapy was given to patients who had a minimum of two vasoactive infusions and had received 50 mL/kg of resuscitation fluids. Nichols *et al*.[12] reported lower numbers of vasopressor and inotropes used, [median 2 (range 1-2)]. In their study, corticosteroids were given to patients with continuous need for catecholamine infusion for 6 or more hours following initial fluid resuscitation of ≥ 60 mL/kg of crystalloid and/or colloid solutions. The cut-off for starting the corticosteroid therapy was not the amount of catecholamine used, but the duration of catecholamine infusion. Nichols *et al.*[12] also showed the catecholamine included dopamine > 5 μg/kg/min, vasopressin, and any dose of dobutamine, epinephrine, norepinephrine, phenylephrine, or milrinone.

We noted that corticosteroid therapy was given without cortisol level examinations, in accordance with the Surviving Sepsis Campaign guidelines which state that in catecholamine-resistant shock, hydrocortisone administration should be given immediately without cortisol level examination. Nichols *et al.*[12] also showed the same statement and in contrast, Casartelli *et al.*[14] concluded that the cortisol assay should be used in deciding whether or not to give corticosteroids in septic shock.

The most commonly used corticosteroid in our study was hydrocortisone (10/12). Similarly, a previous study reported that 78% of subjects were prescribed hydrocortisone, 16% methylprednisolone, and 6% dexamethasone.[9] However, another previous study reported that 53% of subjects were prescribed hydrocortisone, 29% dexamethasone, 14% methylprednisolone, and 4% prednisolone.[15]

Gibbison *et al*.[16] also mostly used hydrocortisone as corticosteroid of choice to treat catecholamine-resistant septic shock, since the advantages of hydrocortisone are increased capillary permeability and cardiovascular activity. Furthermore, hydrocortisone has the lowest risk of side effects such as hyperglycemia, severe infection, and gastrointestinal bleeding compared to other corticosteroids. Hydrocortisone administration may affect cortisol assay results in catecholamine-resistant septic shock, while dexamethasone administration does not.[13]

We noted hydrocortisone loading doses of 2mg/kg, followed by 2-50 mg/kg/d infusion dose. The initial loading dose was similar to that reported by Menon *et al.*,[11] who used a hydrocortisone dose of 2 mg/kg. Their hydrocortisone infusion dose of 1 mg/kg was given every 6 hours and weaned to every 8 hours until all vasoactive infusions had been discontinued for 12 hours. Nichols *et al.*[12] reported an initial hydrocortisone dose of ≥ 50 mg/m^2 (or ≥ 1mg/kg) followed by a dose ≥ 50mg/m2/d (or ≥ 1mg/kg/d).

The median duration of corticosteroid usage in our subjects was 2 (range 1-7) days, with 11/12 patients still receiving corticosteroids when they died. The maximum duration was similar to the Menon *et al.* study,[11] in which corticosteroids were given for a maximum of 7 days to prevent adrenal suppression. However, Nichols *et al.*[12] reported a median of 4 (range 2-4) days corticosteroid therapy in the group with random cortisol level (rSTC) < 18 μg/dL and 4 (range 2-5) days in the group with rSTC ≥ 18 μg/dL. Atkinson *et al.*[9] also showed a higher median duration of corticosteroid use of 5 (range 3-7) days.

In our study, the mortality rate was high (100%), with a median LOS of 3 (range 1-9) days. The high mortality rate may have been caused by the extreme severity of illness among subjects, as indicated by the high use of vasopressor and inotropic agents [median 3 (range 2-4) types] and the high use of mechanical ventilation in 100% of subjects. Menon *et al.*[11] noted a 2% mortality in patients receiving corticosteroid therapy, with PICU median LoS of 8.3 (range 3.7-15.0) days. The median PELOD score of their corticosteroid group was 6 (range 4-9), with 65.2% mechanical ventilation use. According to Dewi Metta *et al.*[20], PELOD scores at 20 increasing mortality to 50%. Nichols *et al.*[12] showed a 24% mortality rate among septic shock patients in the stress dose hydrocortisone therapy group, with median LoS of 10 (range 5-20) days. The median *Pediatric Risk of Mortality* (PRISM) III score at 12 hours was 16 (range 10-12) and 88% of subjects were mechanically ventilated. Vineet Popli *et al.*[21] showed *Pediatric Risk of Mortality* (PRISM) III had a biphasic effect on the length of stay (LOS). Their study showed length of stay increased with increasing PRISM III score up to the score of 14; while score of 19, length of stay decreased gradually because an increasing severity of illness as the mortality reaches almost 100%.

Some guidelines recommend the use of corticosteroids in catecholamine-resistant shock, however, we noted no benefit to such therapy, as 100% of the patients who received corticosteroids died. Menon *et al.*[11] stated that there were no statistically significant differences in outcomes or adverse events between the hydrocortisone and placebo groups. Furthermore, Nichols *et al.*[12] stated that stress dose hydrocortisone therapy in children with catecholamine-dependent septic shock was associated with worst outcomes. In addition, 2 previous studies reported that adjunctive corticosteroid therapy in severe pediatric sepsis showed no definitive improvement.[9,15] Also, a pediatric meta- analysis by Menon *et al.*[17] showed no benefit of corticosteroids for treating shock.

Despite the lack of convincing evidence, a Canadian survey revealed that almost all pediatric intensivists (91.4%) would administer corticosteroids to patients in persistent shock who had received 60 mL/kg of fluid and were on two or more vasoactive medications.[18] Possible rationales for the use of corticosteroids in sepsis are beneficial pharmacologic effect on the cardiovascular system and anti-inflammatory properties. Nonetheless, high dose corticosteroid administration in septic shock has been associated with higher infection rates, such as disseminated candidiasis and hospital- acquired pneumonia.[19] Other potential side effects include hyperglycemia, bleeding, critical illness associated neuropathy/myopathy, and hypernatremia.[15,19] Corticosteroid use was also associated with suppression of genes corresponding to adaptive immunity.[8]

In conclusion, we find that a small proportion of pediatric septic shock patients received corticosteroid therapy, mostly hydrocortisone. The mortality rate of patients who received corticosteroids is 100% and their LoS is short. Corticosteroids do not seem to have beneficial results in our sepsis patient population.

Conflict of Interest

None declared .

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Original Article

Factors associated with insulin-like growth factor-1 in children with thalassemia major

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Abstract

Background Septic shock remains a major cause of mortality Background Insulin-like growth factor-1 (IGF-1) deficiency is the major cause of growth disorders and delayed puberty in children with thalassemia. Hence, identifying factors contributing to IGF-1 deficiency in thalassemia is of importance.

Objectives To evaluate the correlation between IGF-1 level and nutritional status, ferritin, pre-transfusion hemoglobin, thyroid, as well as alanine transaminase levels.

Methods We conducted a study in children aged 2 to 18 years with thalassemia major who visited outpatient clinics at two hospitals in Indonesia, Dr. Sardjito Hospital, Yogyakarta and Dr. Moewardi Hospital, Surakarta, Central Java, from July to December 2015. Clinical, laboratory, and demographic data were reviewed from medical records. IGF-1 levels were measured using an immunochemiluminiscent method.

Results A total of 48 children were recruited into the study. Subjects mean IGF-1 level was 109.28 (SD 90.26) ng/mL. Seventy-five percent of the children had IGF-1 level < -2SD. Subjects mean ferritin, pre-transfusion hemoglobin and ALT levels were 3.568 (SD 2131.31) ng/mL; 7.97 (SD 0.85) g/dL and 49.7 (SD 43.1), respectively. Most of the children (91.7%) was eutyroid, with a mean of TSH and FT4 level was 2.7 (SD 1.5) nmol/L and 12.3 (SD 7.1) μIU/ml, respectively. Ferritin level had no significant correlation with IGF-1 level (r=-0.794; P=0.431). However, a strong, positive correlation was documented between pre-transfusion hemoglobin level and IGF-1 level (r=2.380; P=0.022). Multivariate linear regression analysis revealed that factors with significant correlations to IGF-1 level were pre-transfusion hemoglobin level <8 g/dL (β=-0.090; 95%CI -0.002 to 0.182; P=0.056) and undernutrition (β=0.077; 95%CI 0.045 to 0.109; P<0.001).

Conclusion Low pre-transfusion hemoglobin level and undernutrition are significantly correlated to low IGF-1 level in children with thalassemia major. **[Paediatr Indones. 2019;59:72-8; doi: http://dx.doi.org/10.14238/pi59.2.2019.72-8]**.

Keywords: *thalassemia major; insulin-like growth factor-1; undernutrition*

Thalassemia is a critical health problem in children since it leads to growth and developmental disorders.[1] In 1994, The *World Health Organization* (WHO) reported that 5.2% of the world's population had the thalassemia trait and that increased to 7% in 2001.[2] Each year, there are about 300,000 infants born with thalassemia major worldwide. In Indonesia, the incidence of β-thalassemia, α-thalassemia, and hemoglobin (Hb) E carriers are 10%, 1.2 to 11%, and 1.5 to 36%, respectively.[3]

Due to medical advances in thalassemia management, most patients achieve normal growth in their first decade. However, significant growth disorders and delayed puberty may occur in adolescence,[4] because of insulin-like growth factor-1 (IGF-1) deficiency. A multicenter study conducted in Italy and Qatar reported that 67% of thalassemic adults had IGF-1 deficiency (IGFD).[5] In addition, an Iranian study showed lower mean IGF-1 levels in children with thalassemia [61.33

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(SD 67.64) ng/mL] compared to healthy children [126.93 (SD 156.7) ng/mL].[6] Several factors contributing to the reduction of IGF-1 synthesis include transfusion-induced iron overload, chronic anemia, undernutrition, impaired thyroid function, and high levels of alanine transaminase (ALT).[7] Hence, we aimed to assess such factors for associations with low IGF-1 levels in children with thalassemia major.

Methods

We conducted a retrospective study in outpatient clinics of two hospitals in Indonesia, namely, Dr. Sarjito Hospital, Yogyakarta and Dr. Moewardi Hospital, Surakarta, Central Java, from July to December 2015. We recruited children with thalassemia major aged 2 to 18 years, who were diagnosed based on Hb electrophoresis results. Those who had complete medical records, including ferritin and pre-transfusion hemoglobin levels over the past year, thyroid-stimulating hormone (TSH), free thyroxine (FT4), and ALT levels, were included in the study. We obtained written informed consent from subjects' parents or guardians.

We performed history-taking and medical record reviews to collect the following data: age at diagnosis of thalassemia, Hb electrophoresis results, as well as mean ferritin, pre-transfusion hemoglobin, TSH, FT4, and ALT levels over the past year. The nutritional status were defined by mid upper arm circumference (MUAC), which was measured at the midpoint between the tip of the shoulder and the tip of the elbow (olecranon process and the acromium), and classified according to the classification of Frisanchoas as followed: below adequate ($\leq 5^{th}$ percentile), adequate (between 5^{th} and 95^{th} percentile), and above adequate ($\geq 95^{th}$ percentile). The IGF-1 levels were measured at *Prodia Laboratory* Surakarta, by an automated chemiluminescence immunoassay (*Immulite®2000*), using a solid-phase, enzyme-labeled chemiluminescent immunometric assay. A reference range study performed with *DPC's IMMULITE IGF-*I kit (**Table 1**).[8] The blood was collected at least a week before blood transfusions to avoid elevation of IGF-1 levels due to the transfusion.

Data on the characteristics of subjects were summarized as proportion, mean, or median, where appropriate. We performed a simple linear regression analysis to evaluate for correlations between IGF-1 level and nutritional status, mean ferritin level, mean pre-transfusion hemoglobin level, thyroid function, and ALT level. Pearson's/Spearman's correlation coefficient was used to analyze the strength of linear relationships between variables. The decision in hypothesis testing was based on the significance level of $P<0.05$.[9] The data were analysed using *SPSS 15* software. This study was approved by the Health Research Ethics Committee of the Universitas Gadjah Mada Faculty of Medicine, Public Health and Nursing, Yogyakarta, Indonesia.

Table 1. IGF-I pediatric reference ranges (ng/mL)[8]

Age, year	Median	Central 95% range	0.1 percentile
1	134	55-327	33
2	125	51-303	31
3	119	49-289	30
4	118	49-283	29
5	119	50-286	30
6	124	52-297	31
7	134	57-316	34
8	148	64-345	39
9	169	74-388	46
10	200	88-452	55
11	247	111-551	70
12	315	143-693	91
13	395	183-850	118
14	462	220-972	143
15	486	237-996	157
16	452	226-903	152
17	376	193-731	132
18	308	163-584	112

Results

A total of 48 patients were recruited into the study, 12 children from Dr. Sarjito Hospital and 36 children from Dr. Moewardi Hospital. Subjects' characteristics are presented in **Table 2**. The number of males and females was similar and subjects had a mean of age was 11 (SD 3.6) years. Their mean duration of thalassemia diagnosis in this study was 7.65 (SD 3.60, range 1.2-14.5) years. Subjects mean ferritin level in the past year was 3,568 (SD 2131.3)

ng/mL, with a range of 566ng/mL to 8,453 ng/mL. The highest pre-transfusion Hb level was 9.8 g/dL while the lowest was 5.7 g/dL, with a mean of 8.0 (SD 0.9) g/dL. Based on MUAC measurements, the majority (64.6%) of children were adequate nutrition and none had severely depleted nutrition. Using the 2007 WHO height-to-age z-score charts, 75% of subjects fell into the short stature category and the rest were normal.[10] Most of the children (91.7%) were euthyroid, with mean TSH and FT4 levels of 2.7 (SD 1.5) nmol/L and 12.2 (SD 7.1) μIU/mL, respectively.

Subjects' mean IGF-1 level was 109.3 (SD 90.3) μg/L, with a range of 25 to 491 ng/mL. The majority of the children (75%) had IGF-1 level of less than -2SD, and the rest ranged between -1SD and -2SD. None of the children had IGF-1 levels above the reference value.

Table 3 shows the simple linear regression analysis. Pre-transfusion Hb levels (r=2.380; 95%CI -21.486 to 37.155; P=0.02) and nutritional status (r=5.031; 95%CI 15.409 to 34.503; P=0.000) had significant positive correlations with IGF-1 level. Increased Hb level strongly and significantly correlated with elevated IGF-1 level (**Figure 1**). The equation

Table 2. Characteristics of subjects with thalassemia major

Characteristics	(N = 48)
Sex, n (%)	
Male	26 (54.2)
Female	22 (45.8)
Mean age (SD), years	11.0 (3.6)
Mean age at diagnosis (SD), years	3.5 (3.1)
Mean time since diagnosis (SD), years	7.7 (3.6)
Mean ferritin level (SD), ng/mL	3,568 (2,131.3)
Ferritin level, n (%)	
<2,000 ng/mL	15 (31.3)
≥2,000 ng/mL	33 (68.7)
Mean pre-transfusion Hb level (SD), g/dL	8.0 (0.9)
Hb level, n (%)	
<8 g/dL	23 (47.9)
≥8 g/dL	25 (52.1)
Nutritional status (based on MUAC), n (%)	
Adequate	31 (64.6)
Moderately depleted	17 (35.4)
Severely depleted	0 (0)
Height (age-adjusted), n (%)	
Average	12 (25)
Short	7 (14.6)
Extremely short	29 (60.4)
Mean serum TSH (SD), nmol/L	2.7 (1.5)
Mean FT4 (SD), μIU/mL	12.2 (7.1)
Thyroid function, n (%)	
Euthyroid	44 (91.7)
Hypothyroid	4 (8.3)
Mean ALT (SD), U/L	49.7 (43.1)

Hb=hemoglobin; MUAC=mid-upper arm circumference; TSH=thyroid-stimulating hormone; FT4=free thyroxine; ALT=alanine transaminase

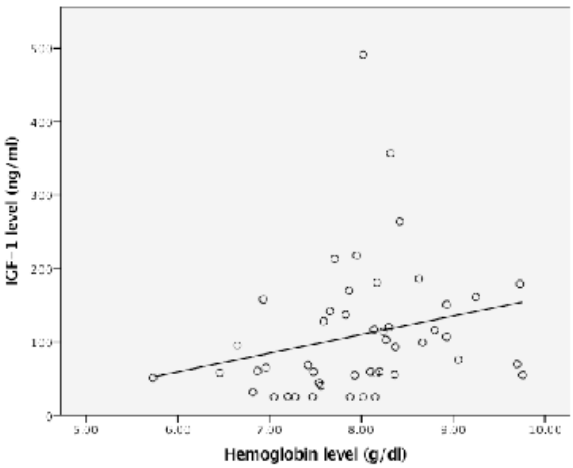

Figure 1. Analysis of IGF- 1 and hemoglobin levels

Table 3. Factors analyzed for correlations with IGF level

Model	Unstandardized coefficients		Standardized coefficients	r	95% CI for B	P value
	B	SE	Beta			
(Constant)	-0.028	0.533		-0.053	-646.924 to -97.408	0.958
Ferritin level	-2.26E-005	0.000	-0.148	-1.048	-0.022 to 0.003	0.301
Hb level	0.898	0.431	0.160	2.380	-21.486 to 37.155	0.022
Nutritional status	0.085	0.017	0.617	5.031	15.409 to 34.503	0.000
Thyroid function	-0.176	0.143	-0.151	-1.231	-116.844 to 46.913	0.226
ALT	0.023	0.117	0.025	0.196	-0.362 to 0.801	0.846

Hb=hemoglobin; ALT=alanine transaminase

from the analysis showed that a decrease of 1 g/dL Hb level would lead to a 0.128 ng/mL decrease in IGF-1 level. Nutritional status had stronger correlation with IGF-1 level, with lower nutritional status significantly associated with lower IGF-1 level (**Figure 2**). The equation indicated that increased nutritional status correlated with elevated IGF-1 level.

The correlation between ferritin and IGF-1 level was negative, but weak and not statistically significant (r=-1.048; 95%CI to 0.22 to 0.003; P=0.301) (**Figure 3**).

Multivariate analyses revealed similar results, with pre-transfusion Hb level (β=0.090; 95%CI -0.002 to 0.182; P=0.056) and nutritional status

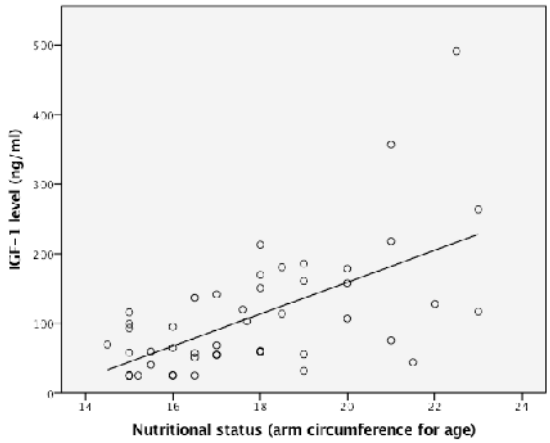

Figure 2. Analysis of IGF- 1 level and nutritional status (by MUAC for age)

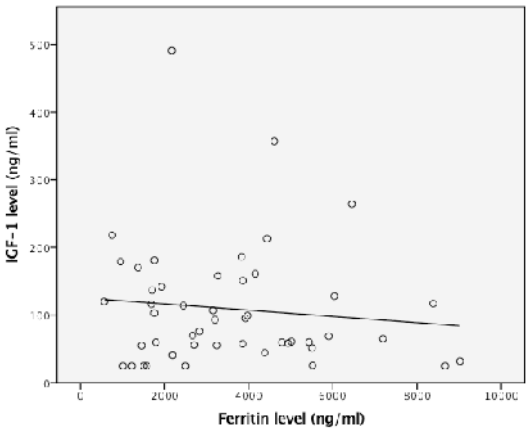

Figure 3. Analysis of IGF- 1 and ferritin levels

Table 4. Multivariate linear regression of independent variables and IGF-1 level

	Model	Unstandardized coefficients		Standardized coefficients	T	95% CI for B	P value
		B	SE	Beta			
1	(Constant)	-0.028	0.533		-0.053	-1.105 to 1.049	0.958
	Ferritin level	-2.26E-005	0.000	-0.148	-1.048	0.000 to 0.000	0.301
	Hb level	0.062	0.051	0.160	1.216	-0.041 to 0.166	0.231
	Nutritional status	0.085	0.017	0.617	5.031	0.051 to 0.118	0.000
	Thyroid function	-0.176	0.143	-0.151	-1.231	-0.466 to 0.113	0.226
	ALT	0.023	0.117	0.025	0.196	-0.214 to 0.260	0.846
2	(Constant)	0.028	0.445		0.062	-0.871 to 0.927	0.951
	Ferritin level	-2.15E-005	0.000	-0.141	-1.044	0.000 to 0.000	0.303
	Hb level	0.060	0.049	0.154	1.216	-0.040 to 0.160	0.231
	Nutritional status	0.084	0.016	0.614	5.102	0.051 to 0.117	0.000
	Thyroid function	-0.173	0.141	-0.149	-1.231	-0.458 to 0.111	0.225
3	(Constant)	-0.127	0.420		0.303	-0.975 to 0.721	0.763
	Hb level	0.081	0.045	0.209	1.802	-0.010 to 0.172	0.079
	Nutritional status	0.079	0.016	0.579	5.005	0.047 to 0.111	0.000
	Thyroid function	-0.219	0.134	-0.188	-1.639	-0.489 to 0.051	0.109
4	(Constant)	-0.171	0.428	-	0.400	-1.033 to 0.691	0.691
	Hb level	0.090	0.046	0.230	1.963	-0.002 to 0.182	0.056
	Nutritional status	0.077	0.016	0.562	4.787	0.045 to 0.109	0.000

Hb=hemoglobin; ALT=alanine transaminase

(β=0.077; 95%CI -0.045 to 0.109; P=0.000) positively correlated with IGF-1 level (**Table 4**). The resulting equation indicated that pre-transfusion hemoglobin level of less than 8 g/dL would decreasing the IGF-1 level of 0.09 μg/L. Meanwhile, for nutritional status, severly depleted in a child with thalassemia major would decreasing the IGF-1 level of 0.077 μg/L.

Discussion

Most thalassemia patients (75%) in our study had low IGF-1 levels [mean 109.3 (SD 90.3) μg/L]. Similarly, a previous study reported that 67% of patients with thalassemia had IGF-1 levels < -2SD.[5] Chronic anemia in thalassemia can cause hypoxia in hepatocytes. This hypoxic state can inhibit protein synthesis in the liver, which leads to increased IGF-binding proteins specific (IGFBPs), especially IGFBP-1. The increased IGFBP-1 may inhibit IGF-1 function by binding to to it and preventing IGF-1 from binding to the IGF-1 receptor (IGF-1R).[9,11]

Repeated blood transfusions in thalassemia major patients may cause elevated levels of free iron in blood serum, which then converts hydrogen peroxide into hydroxide ion (OH-), leading to increased reactive oxygen species (ROS) and oxidative stress.[12] Increased ROS decreases mRNA expression of IGF-1, leading to muscle atrophy, sarcomopenia, wasting, and myopathy. On the other hand, decreased ROS will increase IGF-1 levels, have a positive effect on skeletal muscle protein balance, as well as prevent oxidative damage and other chronic diseases.[13] Our subjects' mean ferritin level was 3,568 (SD 2,131.31) ng/mL, which was higher than reported in an Iranian study [2,962 (SD 1,606) ng/mL].[14] Another study in Malaysia documented that optimal growth in thalassemia major patients can be achieved if their mean ferritin level is less than 2,271 (SD 1804) ng/mL.[15] Several factors can affect ferritin levels in thalassemia patients, including blood transfusion frequency and regularity of iron chelation therapy.[16] We did not analyze factors affecting high ferritin levels in this study because there was no medical record data on the blood transfusion frequency and adherence to iron chelation therapy.

According to *Standards of Care Guidelines for Thalassemia 2009*, Hb levels in children with thalassemia major are ideally maintained at 9-10 g/dL in order to achieve optimal growth.[17] In our study, mean pre-transfusion hemoglobin level was 8.0 (SD 0.9). We noted that Hb level was positively and significantly correlated with IGF-1 level in thalassemia major patients in the univariate analysis (r=2.380; P=0.022), but not in the multivariate analysis.

Micronutrients play a role in growth, protein and DNA synthesis, neurosensory function, immunity, thyroid function, and bone metabolism. Some micronutrients are known to affect IGF-1 level, including magnesium, selenium and zinc. There are two mechanisms involved in the relationship between these nutrients and IGF-1: oxidative stress and inflammation. Micronutrient deficiency increases oxidative stress, free radicals, and oxygen peroxidase production, but decreases antioxidant enzyme expression, which then leads to down-regulated IGF-1 secretion.[18,19]

In our study, nutritional status was assessed by measuring MUAC, due to organomegaly in hepatocytes and/or hemodynamics in thalassemic children. The proportion of subjects in nutritional status categories (64.6% adequate and 35.4% severly depleted) was different from a study conducted in Iran (39.3% adequate and 60.7% severly depleted).[6] Other studies reported the prevalences of severly depleted in thalassemic children to be 64% and 49%, respectively.[6,20] As such, we seemed to have a higher proportion of well-nourished children in our study population. Nonetheless, poor nournishment was significantly correlated to decreased IGF-1 levels. Decreased IGF-1 level in undernourished thalassemic children is caused by elevated interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) secretion in micronutrient deficient conditions. The IL-6 is a major proinflammatory cytokine with a negative effect on muscle function and IGF-1 synthesis.[21]

We noted the prevalence of hypothyroidism in thalassemic children was 8.3%, but this prevalence can vary by country. Previous studies conducted in Indonesia, Iran, and Turkey reported hypothyroidism prevalences of 20%, 6%, and 12.8%, respectively.[22-24]

A previous study found that ALT levels were significantly correlated with IGF-I levels (r=0.26; P=0.05) in thalassemia major patients,[7] contrary

to the findings by De Sanctis *et al.* who found no significant relationship between ALT and IGF-1 levels ($r=0.01$; $P>0.05$).[24]

A limitation of our study that should be considered was that we did not examine IGFBPs levels which can affect IGF-1 levels in the circulation. Moreover, we did not assess factors affecting ferritin levels and nutritional status, such as dietary intake and nutritional interventions.

In conclusion, univariate analysis shows that pre-transfusion hemoglobin levels and nutritional status significantly correlate with IGF-1 levels in children with thalassemia major. Those whose mean pre-transfusion Hb level is less than 8 g/dL and with undernutrition typically have lower IGF-1 levels.

Conflict of Interest

None declared.

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Original Article

Waist circumference, body mass index, and skinfold thickness as potential risk factors for high blood pressure in adolescents

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Abstract

Background The prevalence of hypertension in children and adolescents has increased with the rising obesity epidemic. Recent studies have found that prevalence of hypertension was higher in obese children or adolescents than in the normal weight ones. Anthropometric measurements such as body mass index (BMI), waist circumference, and skinfold thickness have been used as criteria to determine obesity in children and adolescents. Increased waist circumference has been most closely related to increased blood pressure.

Objective To compare waist circumference, BMI, and skinfold thickness as potential risk factors for hypertension in adolescents.

Methods This cross-sectional study was conducted in May 2014 in three senior high schools in Medan, North Sumatera, and included 253 students with normal urinalysis test. All subjects underwent blood pressure, waist circumference, tricep- and subscapular-skinfold thickness (TST and SST), body weight, and body height measurements. The study population was categorized into underweight, normoweight, overweight, and obese, according to four different criteria: waist circumference, BMI, TST, and SST; all variables were analyzed for possible correlations with systolic and diastolic blood pressure.

Results There were significant positive correlations between systolic blood pressure and waist circumference (OR 7.933; 95%CI 2.20 to 28.65; P=0.011) as well as BMI (OR 4.137; 95%CI 1.16 to 14.75; P=0.041). There were also significant correlations between diastolic blood pressure and waist circumference (OR 3.17; 95%CI 1.83 to 5.51; P=0.002), BMI (P=0.0001; OR=3.69), TST (OR 4.73; 95%CI 2.31 to 9.69; P=0.0001), and SST (OR 3.74; 95%CI 2.35 to 5.94; P=0.0001). Multivariate analysis showed that waist circumference was a predictive factor for systolic blood pressure (OR 9.667), but not for diastolic blood pressure.

Conclusion Waist circumference is the strongest, significant, predictive factor for elevated systolic blood pressure; meanwhile BMI, SST, and TST could be predictive factors for elevated diastolic blood pressure. **[Paediatr Indones. 2019;59:79-86; doi: http://dx.doi.org/10.14238/pi59.2.2019.79-86]**.

Keywords:*waist circumference; body mass index; skinfold thickness; blood pressure; adolescents*

Hypertension in children and adolescents has become an important medical problem and a widely-studied topic in recent decades, due to its increased prevalence and resulting sequelae.[1] The prevalence of hypertension in children and adolescents has increased with the rising obesity epidemic. Obesity may eventually lead to metabolic syndrome, consisting of central obesity,

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hypertension, dyslipidemia, and impaired glucose tolerance.[2] Obesity is one of the major health problems often encountered in adolescents.[3] A relationship between obesity and hypertension in children and adolescents has been reported in a wide variety of ethnic groups and races, with higher blood pressure or a higher prevalence of hypertension in obese children and adolescents compared to normal weight ones.[4-6] Body mass index (BMI) is a standard usually used to identify the state of obesity in children and adolescents, but it does not describe the overall levels of body fat in an individual. Because of the limitations of BMI, waist circumference has been used to assess body fat. Compared with BMI, waist circumference is a better index to screen for metabolic abnormalities.[7-9]

Skinfold thickness is an anthropometric parameter that can be used to determine body fat stores and their distribution; central body fat can be measured from the subscapular skinfold thickness (SST) and peripheral body fat can be measured from the triceps skinfold thickness (TST). Skinfold thickness was associated with blood pressure in children and adolescents.[10] Waist circumference is an important method to diagnose central obesity in clinical practice, because of its accuracy and simple technique.[8,11] Waist circumference and its variants, such as the ratio of waist and hip circumference and ratio of waist circumference to height, have been frequently used to assess central obesity, but mainly in adults. Waist circumference has also been used by the International Diabetes Federation (IDF) as a criteria to determine central obesity in adolescents with metabolic syndrome.[12] This study was done to compare waist circumference, BMI, and skinfold thickness as predictive factors of hypertension in adolescents.

Methods

This study was a cross-sectional study to compare waist circumference, body mass index, TST, and SST as predictive factors of high blood pressure in adolescents. This study was conducted in May 2014 at three private senior high schools in Medan, North Sumatera. The inclusion criteria were students aged 15 to 17 years whose parents or guardians allowed them to undergo urinalysis examination, anthropometric measurements such as height, weight, skinfold thickness, and waist circumference, as well as blood pressure examination. The exclusion criteria were students who were taking anti-hypertensive drugs or other drugs which could affect blood pressure, or those likely to suffer from secondary hypertension, which was screened by urinalysis results of proteinuria $\geq +2$, hematuria, or nitrite positive.

This study was approved by the Research Ethics Committee of the University of Sumatera Utara Faculty of Medicine. Subjects' parents or guardians provided written, informed consent. After informed consent was obtained, students underwent history-taking to obtain basic demographic data, as well as their history of the disease and drug use. The simple urinalysis examination was done using Verify® urine dipsticks. Those with proteinuria, hematuria, and/or positive for nitrites were excluded. Students who met the inclusion criteria underwent anthropometric measurements of weight, height, TST, SST, and waist circumference.

Body weight was measured in kilograms using Chinese-made *Camry*® scales, with a measurement scale up to 100 kg and precision of 0.1 kilograms. Height was measured in centimeters using a stadiometer to the nearest 0.1 centimeter. Skinfold thickness was measured in millimeters with an *Accu-measure*® skinfold calliper made in Germany, with an accuracy of 0.1 millimeters. Skinfold thickness measurements were carried out three times and averaged. Based on skinfold thickness, subjects were categorized as underweight for below the 50th percentile, normoweight for 50th to 85th percentile, overweight for >85th to 90th percentile, or obese for above the 90th percentile. Waist circumference was measured with a non-elastic measuring tape at the midpoint between adjacent last ribs and the peak of the iliac crest, at the end of a normal expiration, with the subject standing upright, feet together, arms at the sides, and wearing thin clothing, with a precision of 0.1 centimeters. Measurements were done twice, then averaged if the difference was less than 1 centimeter. If the difference exceeded 1 centimeter, the measurement was repeated. Using waist circumference, subjects were categorized as underweight for below 50th percentile, normoweight for 50th to 85th percentile, overweight for >85th to 90th percentile, and obese for above 90th percentile. Waist circumference and skinfold thickness percentiles for children and adolescents were based on data from the

4th National Health and Nutrition Examination Survey (NHANES IV).[13]

Blood pressure was measured after resting at least 5 minutes, with the patient in the sitting position, the right arm placed at the heart level, using a *NOVA®* mercury sphygmomanometer. Measurements were performed three times, then averaged. Systolic blood pressure was determined at the Korotkoff I sounds and diastolic blood pressure was set when the Korotkoff sounds disappeared. Blood pressure was classified according to the Task Force on High Blood Pressure Education Program as normal if less than the 90th percentile, prehypertension when between 90th and 95th percentiles, and hypertension when above the 95th percentile, according to age, sex, and height.[11] Students who had elevated blood pressure were recommended to be monitored regularly in order to evaluate the need for anti-hypertensive drugs or other interventions.

Subjects' BMIs were calculated using the results of weight and height measurements. BMI was classified according to age and sex as underweight for below 50th percentile, normoweight for 50th to 85th percentile, overweight for >85th to 95th percentile, and obese for above the 95th percentile.[13]

Data were analyzed using *SPSS statistical software version 19.0* with a significance level of $P<0.05$ and 95% confidence interval (CI). Descriptive data were mean, percentage, and standard deviation. Fisher's exact and Chi-square tests were used to analyze the relationships between waist circumference, BMI, and skinfold thickness with systolic and diastolic blood pressure. Multivariate logistic regression with the enter method was used to further analyze for possible relationships between waist circumference, BMI, and skinfold thickness with blood pressures.

Results

Of 393 students at three senior high schools who met the inclusion criteria, 140 students refused to join the study. Of the 253 students who were willing to join, 5 students had hematuria, but after re-analysis, we discovered that these 5 students were menstruating girls, so the urinalysis results were considered to be false positive, and the 5 were included in the study. Hence, the final sample numbered was 253 students.

Subjects' mean age was 16.56 years. Only 18 respondents (7.1%) had a known parental history of hypertension. The demographic characteristics of the study population are shown in **Table 1**.

Table 1. Demographic characteristics of the study population

Demographic characteristics	(N=253)
Sex, n (%)	
Male	129 (51)
Female	124 (49)
Mean age (SD), years	16.56 (0.67)
Birth weight, n (%)	
≤ 2,500 grams	(0.4)
> 2,500 grams	59 (23.3)
Forgot/Unknown	193 (76.3)
Parental history of hypertension, n (%)	
Unknown	112 (44.3)
No	123 (48.6)
Yes	18 (7.1)

Subjects were categorized as underweight, normoweight, overweight, or obese based on their waist circumference, BMI, TST, and SST measurements. Blood pressure was classified into normal and increased (prehypertension and hypertension), respectively, for systolic and diastolic blood pressures. The anthropometric and hemodynamic characteristics of study population are shown in **Table 2**.

With regards to waist circumference, most respondents were categorized as underweight (65.2%). However, the majority of respondents were categorized as normoweight based on BMI examination (50.2%). Using TST and SST measurements, most respondents were categorized as normoweight (75.1%) and (48.2%), respectively].

Blood pressure examinations revealed that 9 subjects (3.6%) had elevated systolic blood pressure and 48 subjects (19%) had elevated diastolic blood pressure. Fisher's exact test revealed a significant correlation between waist circumference and systolic blood pressure ($P=0.011$). The OR value was 7.933, meaning adolescents with waist circumference categories of overweight and obese had an increased risk of elevated systolic blood pressure by 7.933 times compared to the underweight and normoweight categories. We also found a significant correlation between BMI and elevated systolic blood pressure (OR 4.137; $P=0.041$). This OR indicates that

adolescents with BMI categories of overweight and obese had an 4.137 times increased risk of elevated systolic blood pressure compared to underweight and normoweight adolescents. However, SST and TST had no significant correlations with systolic blood pressure (P>0.05), as shown in Table 3.

Table 2. Anthropometric and hemodynamic characteristics of the study population

Anthropometric and hemodynamic characteristics	(N = 253)
Waist circumference, n (%)	
Underweight	165 (65.2)
Normoweight	73 (28.9)
Overweight	8 (3.2)
Obese	7 (2.8)
Body mass index, n (%)	
Underweight	85 (33.6)
Normoweight	127 (50.2)
Overweight	26 (10.3)
Obese	15 (5.9)
Tricep skinfold thickness (TST), n (%)	
Underweight	28 (11.1)
Normoweight	190 (75.1)
Overweight	14 (5.5)
Obese	21 (8.3)
Subscapular skinfold thickness (SST), n (%)	
Underweight	1 (0.4)
Normoweight	122 (48.2)
Overweight	61 (24.1)
Obese	69 (27.3)
Systolic blood pressure (SBP), n (%)	110.15
Mean (SD), mmHg	(7.99)
Range, mmHg	88-128
Normal	244 (96.4)
Increased (prehypertension and hypertension)	9 (3.6)
Diastolic blood pressure (DBP), n (%)	
Mean (SD), mmHg	73.20 (6.55)
Range, mmHg	60-95
Normal	205 (81)
Increased (prehypertension and hypertension)	48 (19)

Statistical analyses revealed significant correlations between waist circumference and diastolic blood pressure (OR=3.17; P=0.002), BMI and diastolic blood pressure (OR=3.69; P=0.0001), SST and diastolic blood pressure (OR=4.73; P=0.0001), as well as TST and diastolic blood pressure (OR=3.74; P=0.0001) (**Table 4**).

Multiple logistic regression analysis with the enter method revealed that the only independent variable that could be used to predict systolic blood pressure was waist circumference. The OR value obtained from the multivariate equation was 9.667, which means that the risk of elevated systolic blood pressure in adolescents with an overweight/obese waist circumference was 9.667 times higher than in adolescents with an underweight/normoweight waist circumference, as shown in **Table 5**.

Multiple logistic regression with the enter method also revealed that the independent variables that could be used to predict elevated diastolic blood pressure were BMI, SST, and TST [(OR 2.808, P=0.013,); (OR 3.377, P=0.008); and (OR 2.729, P=0.022), respectively] (**Table 6**).

Discussion

In recent years of routine blood pressure checks, the prevalence of hypertension in school-aged children has increased from 2% to 5%.[11,14,15] The 2007 Indonesian Ministry of Health Survey (*Riset Kesehatan*

Table 3. Correlations between waist circumference, BMI, TST, and SST with systolic blood pressure

Variables	SBP		OR	95% CI	P value
	Normal	Increased			
Waist circumference, n (%)					
Overweight and obese	12 (80)	3 (20)	7.933	2.20 to 28.65	0.011
Underweight and normoweight	232 (97.5)	6 (2.5)			
BMI, n (%)					
Overweight and obese	37 (90.2)	4 (9.8)	4.137	1.16 to 14.75	0.041
Underweight and normoweight	207 (97.6)	5 (2.4)			
SST, n (%)					
Overweight and obese	123 (94.6)	7 (5.4)	3.312	0.7 to 15.63	0.173
Underweight and normoweight	121 (98.4)	2 (1.6)			
TST, n (%)					
Overweight and obese	32 (91.4)	3 (8.6	3.114	0.82 to 11.88	0.113
Underweight and normoweight	212 (97.2)	6 (2.8)			

Table 4. Correlations of waist circumference, BMI, TST, and SST with diastolic blood pressure

Variables	SBP		OR	95% CI	P value
	Normal	Increased			
Waist circumference, n (%)					
Overweight and obese	7 (46.7)	8 (53.3)	3.17	1.83 to 5.51	0.002[a]
Underweight and normoweight	198 (83.2)	40 (16.8)			
BMI, n (%)					
Overweight and obese	21 (51.2)	20 (48.8)	3.69	2.32 to 5.89	0.0001[b]
Underweight and normoweight	184 (86.8)	28 (13.2)			
SST, n (%)					
Overweight and obese	90 (69.2)	40 (30.8)	4.73	2.31 to 9.69	0.0001[b]
Underweight and normoweight	115 (93.5)	8 (6.5)			
TST, n (%)					
Overweight and obese	17 (48.6)	18 (51.4)	3.74	2.35 to 5.94	0.0001[b]
Underweight and normoweight	188 (86.2)	30 (13.8)			

[a]Fisher's exact, [b]Chi-square

Table 5. Multiple logistic regression analysis of waist circumference, BMI, TST, and SST with systolic blood pressure

Variables	Coefficient	OR	95%CI	P value
1st analysis				
Waist circumference	1794	6.011	0.799 to 45.204	0.081
BMI	0.609	1.839	0.316 to 10.718	0.498
SST	0.710	2.033	0.328 to 12.599	0.446
TST*	-0.235	0.791	0.111 to 5.618	0.815
Constant	-4.153	0.016		0.000
2nd analysis				
Waist circumference	1.682	5.375	0.901 to 32.066	0.065
BMI	0.573	1.773	0.311 to 10.097	0.519
SST	0.667	1.948	0.324 to 11.727	0.467
Constant	-4.147	0.016		0.000
3rd analysis				
Waist circumference	1.964	7.124	1.469 to 34.557	0.015
TST	0.327	2.318	0.431 to 12.470	0.841
Constant	-4.147	0.016		0.000
4th analysis				
Waist circumference	2.269	9.667	2.152 to 43.430	0.003
Constant	-3.655	0.026		0.000

*not analyzed because P>0.05

Table 6. Multiple logistic regression analysis of waist circumference, BMI, TST, and SST with diastolic blood pressure

Variables	Coefficient	OR	95%CI	P value
1st analysis				
Waist circumference*	0.208	1.232	0.334 to 4.574	0.754
BMI	0.995	2.704	1.155 to 6.330	0.022
SST	1.221	3.390	1.385 to 8.298	0.008
TST	0.955	2.599	1.047 to 6.451	0.039
Constant	-2.692	0.068		0.000
2nd analysis				
BMI	1.032	2.808	1.241 to 6.352	0.013
SST	1.217	3.377	1.380 to 8.266	0.008
TST	1.004	2.729	1.159 to 6.423	0.022
Constant	-2.692	0.068		0.000

*not analyzed because P>0.05 for all

Dasar/RISKESDAS) noted a hypertension prevalence of 8.4% in adolescents aged 15 to 17 years.[16] In China, the prevalence of hypertension and prehypertension increased dramatically between 1991 and 2004, with an average increase of 6.38% in children and 8.31% in adolescents.[17] The prevalences of hypertension in adolescents in Turkey was 4.4%,[18] while that in Tunisia, Northern Africa was 39.8%.[19]

We categorized normal vs. increased blood pressure according to systolic and diastolic blood pressure measurements. The prevalence of hypertension and prehypertension by systolic blood pressure was 3.6%, and that defined by diastolic blood pressure was 19%. The overall prevalence of hypertension and prehypertension that defined both by systolic and diastolic blood pressure was 2.4%.

An early symptom of hypertension due to obesity is isolated systolic hypertension (ISH), which is an increase in systolic blood pressure without a subsequent increase in diastolic blood pressure. The term ISH is often used in adults, in which the primary mechanism is due to increased arterial stiffness caused by atherosclerotic vascular disease.[20-22] In obese children, ISH may be caused by hyperactivity of the sympathetic nervous system, which can increase the activity of the renin-angiotensin system, leading to an increase in arterial stiffness by lowering the content of elastin and increasing collagen in the arterial wall. Proliferation of muscle cells in the arterial wall increases arterial thickness and stiffness, and decreases arterial contractility.[21,22] Previous studies have found the prevalence of ISH to be approximately 50% in obese children and 30% in normoweight children. A US study on screening for hypertension and obesity in school children found the prevalence of ISH to be about 95%.[20]

In our study, of nine students with increased systolic blood pressure, four students (44.4%) had ISH. Of students with ISH, one student met the overweight criteria by SST, and one student met the overweight criteria based on BMI and TST, but met the obese criteria based on subscapular skinfold thickness. In addition, one student met the overweight criteria based on waist circumference and SST, while one student met the normoweight criteria based on all parameters.

Chiolero *et al*. in the Seychelles, a developing country in Africa with a predominantly middle-class population, reported that overweight and obese (according to BMI) subjects aged 5 to 16 years had increased blood pressure, in 18% of boys and 26% of girls.[23] Another study in Egyptian adolescents aged 11 to 19 years by Abolfotouh et al. also noted a significant relationship between BMI and increased blood pressure, with OR 2.18.[24] In our study subjects who were categorized as overweight and obese based on BMI, 9.8% had increased systolic blood pressure and 48.8% had increased diastolic blood pressure, with significant relationships between overweight/obese status and increased systolic (OR 4.137) as well as diastolic (OR 3.69) blood pressures.

Aboulfotouh *et al.* in Egypt found a significant association between waist circumference and increased blood pressure, with OR 3.14.[24] Also, Guimares *et al.* found significant associations between waist circumference and systolic blood pressure (PR 1.8; 95%CI 1.0 to 3.0; P=0.036) as well as diastolic blood pressure (PR 1.4; 95%CI 0.8 to 0.24; P=NS), in adolescents aged 11 to 18 years in Salvador, Brazil.[25]

In our overweight and obese subjects based on waist circumference, 20% had increased systolic blood pressure, and 40% had increased diastolic blood pressure. There were significant correlations between higher waist circumference and increased systolic blood pressure (OR 7.933; 95%CI 2.20 to 28.65) as well as diastolic blood pressure (OR 3.17; 95%CI 1.83 to 5.51). Aboulfotouh *et al.* also found that waist circumference had a more significant correlation with blood pressure (OR 3.14; 95%CI 1.67 to 5.94) than did BMI (OR 2.18; 95%CI 1.38 to 3.44). Waist circumference is thought to reflect central obesity while BMI reflects overall obesity.[24] However, Guimaraes et al. found that BMI had a more significant correlation to increased systolic and diastolic blood pressures compared to waist circumference.[25] In our study, the relationship between waist circumference and systolic blood pressure was stronger compared to BMI, whereas for diastolic blood pressure, BMI had a more significant correlation compared to waist circumference.

Kajale *et al.* in India found that waist circumference, BMI and TST had significant relationships with systolic and diastolic blood pressures in children and adolescents.[26] Also, Freedman *et al.* in the Bogalusa Heart Study from 1981 until 1994 found that TST and SST had significant associations with increased blood

pressure. They also found that BMI had a more significant impact on increased blood pressure than did the TST and SST.[27] We found no significant correlations between TST or SST and systolic blood pressure, but both had significant correlations with diastolic blood pressure (OR 3.74; 95%CI 2.35 to 5.94 for TST; OR 4.73; 95%CI 2.31 to 9.69 for SST). In particular, SST is a strong predictive factor for increased diastolic blood pressure in adolescents.

In Indonesia, Allamanda *et al.* conducted a study to investigate if waist circumference could be used as a predictor of hypertension in obese adolescents aged 12 to 17 years. They reported a cut-off value 88.95 centimeters to predict hypertension in adolescents, with sensitivity of 97.8% and specificity of 47.3%.28 We also found that waist circumference could be used as a predictor for increased systolic blood pressure in overweight and obese adolescents (OR 9.667; 95%CI 2.152 to 43.430), but waist circumference was not predictive of increased diastolic blood pressure. However, we could not assign a cut-off value due to the small number of subjects with increased systolic blood pressure.

There were several limitations to this study. We did not analyze other factors that may also influence blood pressure, such as unhealthy living habits. We tried to study birth weight and parental history of hypertension, but much of the data were unknown. Also, although the blood pressure measurement was performed three times and averaged, the measurements were made on the same day. In addition, the number of subjects who met the criteria for overweight and obese was relatively small, such that our results may have differed from previous studies.

Both waist circumference and BMI had significant associations with systolic blood pressure, but waist circumference was more significant as a predictive factor for elevated systolic blood pressure in adolescents. Waist circumference, BMI, and skinfold thickness had significant associations with diastolic blood pressure, but only SST, TST and BMI could be predictors of elevated diastolic blood pressure, with SST as the most significant. Further studies are needed with a larger sample size of overweight and obese adolescents to more accurately assess for relationships between waist circumference, BMI, and skinfold thickness on blood pressure.

Conflict of Interest

None declared.

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Original Article

Diagnostic value of electrocardiography for ventricular septal defects

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Abstract

Background Congenital heart disease (CHD) in children requires attention from medical practitioners, because CHDs that are diagnosed early and treated promptly have good prognoses. Ventricular septal defect (VSD) is the most common type of congenital heart disease.

Objective To compare the accuracy of electrocardiography (ECG) to echocardiography in diagnosing VSD.

Methods This diagnostic study was conducted from November 2013 until July 2015. It involved patients with acyanotic CHDs who were suspected to have VSD at Dr. Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi.

Results Of 114 children screened, 97 were included and analyzed. The frequency of positive VSD was 69.1% based on ECG, and 99% based on echocardiography. There was a significant difference between ECG and echocardiography (P=0.000). However, when small VSDs were excluded, there was no significant difference between the two diagnostic tools [(P=1.000), Kappa value was 0.66, sensitivity was 98.5%, specificity was 100%, positive predictive value (PPV) was 100%, and negative predictive value (NPV) was 50%].

Conclusion There were significant differences between the ECG and echocardiography, for diagnosing VSD. However, if small VSDs were not included in the analysis, there was no difference between the two examinations, suggesting that ECG might be useful for diagnosing VSD in limited facilities hospitals. **[Paediatr Indones. 2019;59:87-91; doi: http://dx.doi.org/10.14238/pi59.2.2019.87-91]**.

Keyword: *acyanotic CHD; VSD; electrocardiography; echocardiography*

Pediatric congenital heart disease (CHD) requires attention from medical practitioners, especially physicians, because early diagnosis of CHD followed by prompt treatment results in improved prognoses. Late detection of congenital heart disease in infants or children delays their treatment, not only causing children to suffer, but prolonging hardship for parents and families. Therefore, diagnosis and management of CHD should be done as early as possible, so that the child survives with a better future.[1]

Although more sophisticated diagnostic tools such as echocardiography for CHD diagnosis exist, the roles of history, physical examination, and diagnostic tools such as electrocardiography (ECG) are not less important. Electrocardiography is a non-invasive, practical, as well as inexpensive diagnostic tool, and is available in a variety of places. However, examinations using an ECG should still be based on history and

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physical examination, because some CHDs present similarly by electrocardiogram.[1] In ventricular septal defects (VSD), hemodynamic changes occur due to an interventricular defect. Due to left-to-right shunt, the left cardiac output changes. Those circumstances lead to left atrial enlargement (LAE) and left ventricular hypertrophy (LVH). If these conditions are continuous, the pressure in the right ventricle will also increase, possibly leading to right ventricular hypertrophy (RVH). Electrocardiography can record the presence of LAE, LVH, and RVH.[2] Therefore, it is important to compare the findings of ECG to those of echocardiography.

In children with prolonged undetected and untreated CHD, 50% may die in the first month of life or 70% may die in the first year of life.[3,4] Echocardiography is the standard diagnostic tool for VSD, but it can be performed only in certain places by experts. Therefore, we aimed to investigate the usefulness of ECG in diagnosing VSD, for early detection and treatment, in order to reduce morbidity and mortality rates. To our knowledge, the diagnostic value of electrocardiography for VSD has never been studied in Indonesia. As such, we aimed to assess the accuracy of electrocardiography in diagnosing ventricular septal defect compared to echocardiography.

Methods

This diagnostic study was conducted at Dr. Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, from November 2013 to July 2015 with primary and secondary data, as well as measurements performed by cross-sectional method. This study compared ECG and echocardiography as the gold standard. The study subjects were the population of children with acyanotic congenital heart disease and suspected VSD who met the inclusion criteria, which were acyanotic CHD presenting with pansystolic murmur on ICS III-IV with or without other murmur, aged 3 months to 15 years, had complete medical records, and parents' willingness to participate in the study. Patients were excluded if their ECG recording could not be read. Primary and secondary data were grouped according to destination and type, then were analyzed by univariate analysis, McNemar test, or association coefficient. The result of electrocardiography would be interpreted by verificator. There were validity test and reliability test among verificators with McNemar analysis. Patients with VSD diagnosis were grouped into small VSD (diameter <3mm), moderate VSD (diameter 3-8 mm), and large VSD (diameter ≥8mm).

This study was approved by the Research Ethics Committee of the Universitas Hasanuddin Medical School.

Results

From November 2013 to July 2015, there were 114 CHD patients who were suspected to have VSD. However, 17 children were excluded resulting in a total of 97 subjects. **Table 1** shows the subjects' characteristics: age, sex, nutritional status, major defect, and type of defect. Of 97 subjects, 50 (51.5%) were female. The mean age of the subjects was 4 years and 15 days, with a range of 3 months to 13 years and 3 months. Subjects' nutritional status types were poorly nourished in 41 (42.3%), undernourished in 36 (37.1%), and good nutrition in 20 (20.6%) subjects. The VSD sizes were small in 28 (28.8%) subjects, moderate in 42 (43.2%) subjects, and large in 27 (28%) subjects. Isolated VSD was found in 82 (84.5%) subjects, while the complex VSD was found in 15 (15.5%) subjects.

Intra-examiner reliability and validity in assessing ECG was analyzed in 10 subjects with McNemar and Kappa tests (**Table 2** and **Table 3**).

Table 1. Study subjects' characteristics

Characteristics	Total (N=97)
Age, years	
Mean (SD)	4.04 (3.59)
Median (range)	3.00 (0.25 – 13.25)
Sex, n (%)	
Female	50 (51.5)
Male	47 (48.5)
Nutritional status, n (%)	
Well-nourished	20 (20.6)
Undernourished	36 (37.1)
Poorly-nourished	41 (42.3)
Defect size, n (%)	
Small VSD	28 (28.8)
Moderate VSD	42 (43.2)
Large VSD	27 (28)
Type of VSD, n (%)	
Isolated	82 (84.5)
Complex	15 (15.5)

Table 2. Analysis of intra-examiner ECG interpretation reliability

	Verificator I		Total
	LVH	Not LVH	
Verificator II			
LVH	6	0	6
Not LVH	0	4	4
Total	6	4	10

Table 3. Analysis of inter-examiner ECG interpretation validity

	Verificator I		Total
	LVH	Not LVH	
Verificator II			
LVH	6	0	6
Not LVH	0	4	4
Total	6	4	10

McNemar analysis revealed no significant differences among examiner readings and the Kappa value was 1. Hence, intra-examiner reliability and validity were sufficient.

Table 3 shows inter-examiner validity in assessing ECG, as analyzed by McNemar and Kappa tests. McNemar analysis no significant differences and the Kappa value was 1. Hence, inter-examiner assessments had a strong degree of conformity.

The diagnostic value of ECG was compared to echocardiography. VSD was detected in 67 (69.1%) subjects using ECG and 96 (99%) subjects using echocardiography (**Table 4**). McNemar analysis revealed a significant difference between the two examinations (P=0.000), with a Kappa value of 0.045, indicating a weak degree of conformity between the two examinations.

After the small VSD subjects were excluded from the analysis, the frequency of VSD occurrence based on ECG was 66 (97.1%) subjects and 67 (98.5%) subjects based on echocardiography. McNemar analysis revealed a no significant difference between the two examinations (P=1.000), with a Kappa value of 0.66, indicating that the degree of conformity between the two examinations was moderate (**Table 5**). For diagnosing VSD, electrocardiography sensitivity was 98.5%, specificity was 100%, positive predictive value was 100%, and negative predictive value was 50%.

Table 4. Analysis of VSD occurrence based on ECG and echocardiography

	Echocardiography		Total
	VSD	Not VSD	
Electrocardiography			
VSD, n (%)	67 (69.1)	0 (0)	67 (69.1)
Not VSD, n(%)	29 (29.9)	1 (1)	30 (30.9)
Total, n(%)	96 (99)	1 (1)	97 (100)

There was one subject in our study with an echocardiogram that showed ASD, and it ECG result confirmed that the subject had no VSD because there was RVH on electrocardiogram recordings. The subject was included in the sample, as this was due to misinterpretation of a murmur.

Discussion

In this study, suspected VSD in acyanotic CHD was more frequent in girls than boys (51.5% *vs.* 48.5%, respectively). Similarly, a previous study in Canada reported a slightly higher CHD incidence in girls (52%).[5] The average age of patients with suspected VSD was 4 years and 15 days, with the highest occurrence in 3-year- olds. In contrast, Shah *et al.* showed that the age of 1 month to 1 year (46.4%) was most common in terms of suspected VSD, while there were only 9.5% in neonates.[6] Neonates were not included in our study criteria since heart murmurs at such an age are difficult to detect because of the high pressure in the right heart chamber. Nonetheless, the average age of the subjects was quite high (4 years and 15 days), indicating that in general, early detection ability was still poor.[1]

The VSD types found in our subjects were isolated VSD (82 subjects, 84.5%) and VSD with other CHDs such as atrial septal defect (ASD), patent

Table 5. Analysis of moderate-large VSD occurrence based on ECG and echocardiography

	Echocardiography		Total
	VSD	Not VSD	
Electrocardiography			
VSD, n (%)	66 (97.1)	0 (0)	66 (97.1)
Not VSD, n(%)	1 (1.4)	1 (1.5)	2 (2.9)
Total, n(%)	67 (98.5)	1 (1.5)	68 (100)

ductus arteriosis (PDA), and transposition of the great arteries (15 subjects, 15.5%). Hariyanto in Padang also observed isolated VSD (16 cases, 45.7%) and VSD with other CHDs [ASD (7 cases, 20%) and PDA (3 cases, 8.5%)].7

The CHD patients mostly poorly nourished (42.3%), however, 20.6% of patients had good nutritional status, and 37.1% had undernourished Likewise, another study in Bandung showed that VSD patients tended to poorly nourished, where 24% of VSD patients had severe malnutrition, 16% had moderate malnutrition, 33% had mild malnutrition, and 27% patients had good nutrition.[8] Malnutrition was mostly found in patients with large VSDs. The presence of pulmonary hypertension was a risk factor related to the occurrence of malnutrition. Patients with increased blood flow to the lung and pulmonary hypertension had an increased chance of malnourishment and stunted growth.[9] Malnourished children are more prone to infection, which further aggravates their condition. Furthermore, anorexia, inadequate nutritional intake, hypoxemia, hyper-metabolic status, acidemia, cation imbalances, reduced peripheral blood flow, chronic decompensated heart disease, malabsorption, protein loss, recurrent respiratory infection, hormonal factors, and genetics may also eventually lead to malnutrition in CHD patients.[10]

In our study, the frequency of VSD based on ECG was 69.1%, while the frequency of VSD based on echocardiography was 99%. Statistical analysis revealed that the two tests were significantly different (P=0.000). In general, ECG capability in diagnosing VSD is not comparable to echocardiography because of the high frequency of small VSDs (28.8%) with normal ECG. Biologically, small VSDs produce normal electrocardiograms, because the light volume overload in the left heart is undetectable in electrocardiogram recordings.[1]

After small VSDs were excluded in our analysis, VSD occurrence based on ECG was 97.1%, while the VSD occurrence based on echocardiography was 98.5%. Statistical analysis revealed no significant difference between the frequencies of positive VSDs from the two examinations. In other words, ECG capability in diagnosing VSD was comparable to echocardiography, if the defect size was moderate-large. ECG sensitivity compared to echocardiography as the gold standard in moderate-large VSDs was 98.5%, which means that ECG could be used to precisely diagnose moderate-large VSDs. ECG specificity in moderate-large VSD was 100%. As such, ECG could be used to rule out the possibilty of moderate-large VSDs. The positive predictive value was 100%, indicating that a positive VSD result based on ECG was 100%. However, the negative predictive value of the ECG for diagnosing moderate-large VSDs was 50%, indicating that possibly half of patients with negative VSD results based on ECG might actually have a VSD. Hence, the benefits of the application for diagnostics lies in the positive predictive value. If a patient is clinically suspected of having VSD with acyanotic CHD and the ECG shows signs of LVH, then it strongly suggests that the patient has a moderate-large VSD and must be treated. The negative predictive value of only 50% means that if the patient's ECG is normal, then the patient should be immediately referred to tertiary care to be further evaluated by echocardiography. Results of this study can only be applied to acyanotic CHD patients with suspected VSD. Acyanotic VSD patients who have pulmonary hypertension should be referred to tertiary care for echocardiography and timely treatment, as pulmonary hypertension can lead to Eisenmenger syndrome.

Biologically, ventricular septal defect shows signs of LVH in electrocardiogram recordings because of volume overload in the left ventricle, while RVH is caused by volume overload in the right heart and pulmonary stenosis is found in ASD.[1] In addition, a subject with moderate VSD by echocardiography but normal electrocardiogram was considered to be a false negative. Conceptually, this VSD with a normal electrocardiography result was because in moderate VSD, significant hemodynamic disturbances occur in defects > 5 mm.[11]

The strength of this study was the large sample size. Determination of the number of samples by using the VSD occurrence frequency is high at around 30%. Electrocardiography and echocardiography results were interpreted by a pediatric cardiologist, followed by verification of their validity and reliability. Moreover, this study was conducted at Dr. Wahidin Sudirohusodo Hospital, which is the national referral hospital in Eastern Indonesia. Therefore, the data are representive of acyanotic congenital heart disease in Eastern Indonesia. A limitation of this study was that the capability of ECG to diagnose VSD

accompanied by cyanotic congenital heart disease was not performed.

In conclusion, there are significant differences between ECG and echocardiography for diagnosing VSD in children with acyanotic CHD. If small VSDs are not included in the analysis, there is no significant difference between the two examinations. As such, ECG may be useful for identifying moderate-large VSDs.

Conflict of Interest

None declared.

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Original Article

Predictors of mortality in children with acute kidney injury in intensive care unit

Umi Rakhmawati, Indah K. Murni, Desy Rusmawatiningtyas

Abstract

Background Acute kidney injury (AKI) can increase the morbidity and mortality in children admitted to the pediatric intensive care unit (PICU). Previous published studies have mostly been conducted in high-income countries. Evaluations of possible predictors of mortality in children with AKI in low- and middle-income countries have been limited, particularly in Indonesia.

Objective To assess possible predictors of mortality in children with AKI in the PICU.

Methods We conducted a retrospective cohort study at Dr. Sardjito Hospital, Yogyakarta. All children with AKI admitted to PICU for more than 24 hours from 2010 to 2016 were eligible and consecutively recruited into the study. Logistic regression analysis was used to identify independent predictors.

Results Of the 152 children with AKI recruited, 119 died. In order to get a P value of <0.25, multivariate analysis was run to degree AKI, ventilator utilization, primary infection disease, multiple organ failure (MOF), and age. Multivariate analysis showed that ventilator use, severe AKI, and infection were independently associated with mortality in children with AKI, with odds ratios (OR) of 19.2 (95%CI 6.2 to 59.7; P<0.001), 8.6 (95%CI 2.7 to 27.6; P<0.001), and 0.2 (95%CI 0.1 to 0.8; P=0.02), respectively.

Conclusion The use of mechanical ventilation and the presence of severe AKI are associated with mortality in children with AKI admitted to the PICU. Interestingly, the presence of infection might be a protective factor from mortality in such patients. **[Paediatr Indones. 2019;59:92-7; doi: http://dx.doi.org/10.14238/pi59.2.2019.92-7]**.

Keyword: *predictor; death; acute kidney injury; PICU*

Acute kidney injury (AKI) might increase morbidity and mortality in children admitted to intensive care units. These critically ill children are often treated for sepsis using nephrotoxic drugs. Renal ischemia might be related to the development of AKI. The incidence of AKI in children varies from 16.7 to 50%, with a 32% mortality rate.[1]

Early detection of mortality predictors in children with AKI is important in order to improve outcomes. Previous studies have identified predictors of mortality in children with AKI, but studies conducted in children from low- and middle-income countries have been limited. An Indian study reported that age, infection, sepsis, shock, heart disease, mechanical ventilation, PRISM score, hypoxia, and coagulopathy were associated with mortality in children with AKI.[2]

To our knowledge, no studies have been published on the predictors of mortality in children

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with AKI treated in Indonesian PICUs, therefore, we aimed to identify such predictors in Dr. Sardjito Hospital, Yogyakarta, Indonesia.

Methods

A retrospective cohort study was conducted to determine mortality predictors in children with AKI in the PICU. All severely ill children aged less than 18 years, diagnosed with AKI, admitted to the PICU at Dr. Sardjito Hospital, Yogyakarta, Indonesia for at least 24 hours in 2010-2016, and who met at least one of the risk, injury, failure, loss, end stage renal failure (RIFLE) criteria, were eligible for the study. The dependent variable was mortality. Independent variables, which were the potential predictors of mortality, were identified based on epidemiological and clinical parameters. These factors included a severe condition as determined by RIFLE criteria, age, gender, nutritional status, length of PICU stay, multi-organ failure, ventilator usage, and primary disease.

The RIFLE criteria were used to classify AKI severity, in which the condition was categorized into injury, failure, loss, or end-stage phase. Nutritional state was assessed using the WHO Z-score curve for children aged ≤5 years old or BMI-for-age for children aged >5 years old.[3] Malnutrition was defined as weight/height Z-scores or BMI-for-age of <-2SD or >+2SD. Multi-organ failure was defined as a state of two or more organ system failures (cardiovascular, respiratory, neurological, gastrointestinal, and hematological) characterized by worsening clinical and laboratory parameters. Primary disease was defined as the patient's major diagnosis recorded in the medical record. For complex conditions, we classified the primary disease based on what was written in the medical record by an intensivist who treated the patient as the main reason for PICU admission. Primary diseases were recorded as nominal data, using the following categories: (1) chronic, in which the primary diagnosis was a chronic disease, such as congenital heart disease or autoimmune disease (2) infection, in which the primary diagnosis was infectious disease, such as pneumonia, sepsis, or meningoencephalitis, and (3) malignancy, in which the primary diagnosis was hematological malignancy or solid tumor, including acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), retinoblastoma, osteosarcoma, neuroblastoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, nasopharyngeal carcinoma, rhabdomyosarcoma, histiocytosis, and nephroblastoma.[4] Data were analyzed using *SPSS 23* software. We used Chi-square bivariate analysis to determine the magnitude and strength of association between independent (predictors) and dependent (mortality) variables. Multivariate analysis was also conducted to determine predictors that were independently associated with mortality. For these analyses, all potential predictive factors were selected, including all variables found to be significant in the univariate analysis ($P<0.25$), and analyzed by multiple logistic regression. Multivariate analysis results are reported as odds ratios.[5]

Preliminary data collection was conducted by looking for ICD-10 code of N.17 (Acute Renal Failure) in patients' medical records starting in 2010. After 2013, data collection was conducted by looking for AKI diagnoses in patients' medical records. For each medical record with an N.17 diagnosis code, the RIFLE criteria were inspected. If the patient met the RIFLE criteria, they were included as research subjects. Patient identity and data were confidential; names and medical record numbers were not included in the study data and only the investigators knew the patient codes.

This study was approved by the Ethics Committee of Medical Research of the Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada/ Dr. Sardjito Hospital, Yogyakarta prior to conducting the research.

Results

A total of 2,630 children were treated in the PICU of Dr. Sardjito Hospital, Yogyakarta, Indonesia for a minimum of 24 hours from 2010 -2016. Among these patients, 152 were diagnosed with AKI according to medical records. Of the 152 children with AKI, 119 (78.3%) died. The baseline characteristics of all the AKI patients are presented in **Table 1**.

Of 10 possible predictors of mortality in children with AKI, the bivariate analysis identified 5 potentially significant associations including age, degree of severity, multi-organ failure, ventilator use,

Table 1. Characteristics of children with AKI

Characteristics	Died (n = 119)	Survived (n = 33)
Median age, years (range)	3 (0-17)	8 (0-15)
Male, n (%)	67 (56.3)	22
Nutritional status, n (%)		
Well-nourished	68 (57.1)	21
Malnourished	29 (24.4)	2
Severely malnourished	15 (12.6)	0
Excessively nourished	7 (5.9)	0
AKI level, n (%)		
Mild-to-moderate (risk)	79 (66.4)	8
Severe (injury, failure, loss, end stage)	40 (33.6)	25
Mean PICU length of stay (SD), days	7.4 (6.4)	7.06 (3.0)
<7 days PICU length of stay, n (%)	74 (62.2)	18

Table 2. Bivariate and multivariate analyses of predictors of mortality in children with AKI

Predictors	Bivariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Age				
≤ 5 years old	1.87 (0.8 to 4.1)	0.11	1.5 (0.5 to 4.4)	0.50
>5 years old				
Gender				
Male	0.64 (0.3 to 1.4)	0.28		
Female				
Multi-organ failure				
Yes	3.39 (0.7 to 15.3)	0.09	2.9 (0.5 to 17.4)	<0.001
No				
AKI severity level				
Mild				
Severe	6.17 (2.5 to 14.9)	0.00	8.6 (2.7 to 27.6)	<0.001
Length of stay				
< 7 days				
≥ 7 days	0.73 (0.3 to 1.6)	0.43		
Ventilator usage				
Yes	15 (6.0 to 37.4)	0.001	19.2 (6.2 to 59.7)	<0.001
No				
Nutritional status				
Well-nourished				
Malnourished	1.03 (0.5 to 2.3)	0.94		
Primary disease				
Infection				
Yes	0.40 (0.2 to 0.9)	0.03	0.2 (0.1 to 0.8)	0.02
No				
Malignancy				
Yes	0.83 (0.1 to 8.2)	1.0		
No				
Chronic disease				
Yes	0.67 (0.2 to 2.3)	0.51		
No				

and lack of infectious primary disease. Multivariate analysis revealed that multi organ failure, the degree of severity, ventilator use, and lack of infection remained independently associated with mortality in children with AKI (**Table 2**).

Discussion

Various criteria have been used to diagnose AKI. The amount of urine production, urinalysis data, blood urea nitrogen (BUN) level, and serum creatinine have been used as laboratory parameters, however, these examinations have low sensitivity and specificity.[1] A Denpasar, Indonesia study reported an incidence of 16.77% of AKI in the PICU, lower than in Cincinnati, Ohio, USA (30%-50%). In addition, a Puerto Rico study noted a 27.4% AKI incidence in the PICU within the first 72 hours of treatment.[1,6-8]

Some studies showed that severe AKI, as determined using pRIFLE criteria, is an indicator of poor prognosis. The Denpasar, Indonesia study found a 32% mortality rate due to AKI,[1] while a Brazilian study found a 24.6% mortality rate, 10 times higher than that of patients without AKI. The mortality rate in patients with various levels of AKI was 5 times higher than that in patients without AKI.[9] The risk of dying in patients with AKI was 10 times higher than in patients without AKI. Furthermore, a study in Spain also found 32.6% mortality due to AKI in the PICU.[10] Moreover, a study in a Brazilian PICU found that 82% of patients with AKI met pRIFLE classification with 15.1% mortality rate.[11]

In our study, the incidence of AKI varied and significantly increased after 2013. This observation might have been because the strict application of RIFLE criteria for diagnosing AKI started that year. The incidence of AKI in 2016 was 40%, which was consistent with previous studies that showed incidences of AKI in low- and middle-income countries (e.g., India, Jordan, and others) ranging from 1 to 58%,[10] and in high-income countries (e.g., USA, others) ranging from 5.4% to 30%.[1,12-15]

In our study, approximately three-quarters of children with AKI died. A Hong Kong study conducted in a PICU reported a mortality rate of 41%.[16] Cabral *et al.* in Portugal showed that severe AKI (injury and failure phases) had a mortality rate of 21% compared to other phases of AKI.[17] In addition, an Indian study found that the injury phase of AKI had the highest mortality (50%) compared to other phases.[13] The mortality rate in our study was higher compared to previously published studies, perhaps due to differences in patient characteristics, risks, types of treatment, and severity of illness.

In our study, more severe AKI was significantly associated with mortality. Multivariate analysis revealed that severe AKI increased mortality risk by 8.6 times compared to mild-to-moderate AKI. This result was consistent with a Jordanian study, which noted an OR of 6.3 (95%CI 5.6 to 7.4; P<0.001).[15] The failure phase of AKI upon PICU admission was reported to be an independent predictor of mortality in the PICU.[15] Also, the mortality rate of AKI patients in the injury and failure phases was twice as high compared to those in the risk phase.[18] The failure phase of AKI represents the most severe form of AKI, which might have been accompanied by severe organ dysfunction and reduced reversibility of health status. Similarly, Soler *et al.* found that the injury and failure phases of AKI, as well as length of stay, were related to morbidity and mortality.[8]

The majority of our patients used mechanical ventilation, which was associated with an increased risk of death by 19.2 times compared to those without ventilator use. This finding was comparable to studies conducted in Jordan, Hong Kong, Brazil and Taiwan, which noted that ventilator use was associated with 6.7 to 80 times higher mortality among children with AKI.[11,13,15,19] The use of endotracheal tubes and mechanical ventilation was associated with increased mortality due to alteration of bacterial colonies in the respiratory tract, aspiration of oropharyngeal secretions, or impaired clearance of lung secretions, all of which subsequently can lead to the development of ventilator-associated pneumonia.[20,21]

Infection was the most common primary disease in our subjects. However, multivariate analysis showed that infection was a protective against mortality in children with AKI. This result was inconsistent, as infections tend to worsen the condition of patients, subsequently leading to death.[17] As such, an explanation might be that our patients with acute infection had no chronic disease or they were in relatively good condition prior to being treated in the PICU, therefore, adequate treatment for acute

infection might HAVE resulted in good recovery or prognosis.

Multivariate analysis revealed no significant association between age and increased risk of death in AKI patients. However, previous studies noted that younger age was associated with mortality,[2,8,19] except for an indian study showed that age more than 5 years was a significant risk factor for severe AKI.[13] Differences of PICU patient characteristics may account for these discrepancies.[15]

No association was found between gender and increased risk of death, consistent with previously published studies.[2,8,18] About half of our AKI patients had wellnourished, nonetheless, malnutrition was also not associated with an increased risk of mortality. Similarly, Imani *et al.* showed that nutrition was not associated with AKI (OR 1.8; 95%CI 1.2 to 2.7).[6] However, a Puerto Rican study found that low body weight was a predictor of AKI mortality.[8] These different results might be due to different characteristics of PICU patients, such as age, type of diseases, and morbidity.

Multivariate analysis also revealed no association between length of stay and increased risk of death, unlike previous studies where AKI increased the risk of longer hospitalization in PICU (OR 3.73; 95%CI 1.89 to -7.38),[8] and longer length of stay increased mortality, especially among those with multi-organ failure.[15]

In our study, multi-organ failure was associated with a three-fold increase in mortality in children with AKI. This finding was in general agreement with a previous study, which stated that children with AKI who developed multi-organ failure had a survival rate of 43% (6/14).[22] Multi-organ failure also served as an independent risk factor of AKI mortality in the PICU (OR 3.21; 95%CI 2.08 to 4.94).[2] In addition, multi-organ failure increased mortality risk by 10-57%.[23] As such, multi-organ failure was a significant variable for the development of severe AKI.[8,13]

To our knowledge, this study was the first from Indonesia to report on predictors of mortality in children with AKI who were admitted to the PICU. Our findings might provide guidelines to prevent mortality in this population. However, there were some limitations to this study. We used a retrospective cohort design to collect data from medical records, in which information bias might have occurred. A selection bias could also occur due to the fact that only patients diagnosed with AKI based on medical records were included in the study.

In conclusion, ventilator usage, severe AKI, multi-organ failure, and lack of infection are associated with mortality in severely ill children with AKI. Our findings suggest that early diagnosis of AKI and identification of those particular mortality predictors in children admitted to the PICU might reduce mortality rates and give better outcomes.

Conflict of Interest

None declared.

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Original Article

Relationship between serum zinc and homocysteine in children with nephrotic syndrome

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Abstract

Background In children, most idiopathic nephrotic syndrome (NS) is a minimal lesion, which responds well to steroids. Hyperhomocysteinemia is pathologic and worsens NS by causing chronic inflammation, leading to glomerular sclerosis. Zinc metalloenzymes are involved in homocysteine metabolism.

Objective To assess for a possible relationship between serum zinc and homocysteine in children with NS.

Methods A cross-sectional study was conducted in children with NS aged 1-18 years, who were admitted to Hasan Sadikin Hospital, Bandung, West Java, from November 2017 - January 2018. Subjects were selected consecutively. Serum zinc and homocysteine were measured in all subjects. Statistical analysis was done with Pearson's correlation test. If the distribution was not linear, the analysis was continued with non-linear regression.

Results There were 23 children who met the inclusion criteria. Mean serum homocysteine and zinc levels were 10.37 (SD 4.11) μmol/L and 51.13 (SD 29.69) μg/dL, respectively. Pearson's correlation analysis showed no linear correlation between them (r coefficient -0.173; P=0.430). However, after adjusting for age and serum albumin level, multiple regression analysis suggested a cubical relationship between serum homocysteine and zinc, using the equation: homocysteine = -4.572 + 0.735 x zinc - 0.0012 x zinc^2 + 0.00005 x zinc^3 x age (months) (R^2 multiple=53.2%; P=0.012). This equation indicates that 53.2% of homocysteine variation was influenced by serum zinc concentration.

Conclusion In childhood NS, homocysteine is not correlated linearly with zinc, but related with cubical model. **[Paediatr Indones. 2019;59:98-103; doi: http://dx.doi.org/10.14238/pi59.2.2019.98-103].**

Keyword: *children; relationship; nephrotic syndrome; homocysteine; zinc*

Nephrotic syndrome (NS) is a common pediatric kidney disease characterized by leakage of protein from the blood into the urine. It remains a major cause for referral to pediatric nephrologists because of the chronicity and the complexities of the disorder.[1] Nephrotic syndrome can be classified into 3 groups: primary or idiopathic, if not accompanied by other systemic diseases, secondary to disease or other systemic conditions, and congenital NS.[1] As many as 90% of NS cases in children aged 1-10 years are idiopathic.[2] Most idiopathic NS in children are minimal lesions which respond well to steroid therapy.[3]

Focal segmental glomerulosclerosis (FSGS) is a further stage of such a minimal lesion.[3] In contrast to the minimal lesions that have not undergone structural changes in light microscopy, focal segmental glomerulosclerosis is characterized by segmental destruction of the glomerular capillaries, accompanied by adhesions formed between the sclerosis segment and Bowman's capsule.[3] As much as 80% of FSGS

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forms are resistant to steroid treatment and will develop into end-stage renal disease (ESRD). [3]

Clinical and epidemiologic studies in the last 20 years have shown a positive correlation between elevated homocysteine (Hcy) levels and ESRD as well as their cardiovascular complications.[4] Laboratory studies have shown that Hcy directly induces glomerular injury, affects glomerular endothelial cells, mesangial cells, and podocytes.[5]

Zinc is an important mineral for the human body and is known to have a role in Hcy metabolism. To date, studies on the effect of zinc on NS have been limited and to our knowledge no study has been done directly correlating zinc and Hcy in pediatric NS.[6] Two zinc metalloenzymes involved in Hcy metabolism are methionine synthase (MS) and betainehomocysteine methyltransferase (BHMT).[7] Both of these enzymes play a role in catalyzing methyl transfer in the homocysteine metabolic process.[8] The purpose of this study was to analyze for a correlation between serum zinc and homocysteine levels in children with NS.

Methods

This cross-sectional study was performed in children with nephrotic syndrome in the Pediatric Nephrology Division, Hasan Sadikin Hospital, Bandung, West Java, from November 2017 to January 2018. The inclusion criteria were children aged 1 to 18 years who were diagnosed with NS during the nephrotic stage. We excluded NS patients with Down syndrome, proliferative blood disorder, hypothyroidism, hyperthyroidism, diabetes mellitus, chronic kidney disease with glomerular filtration rate <60 mL/minute/1.73m^2, severe malnutrition, chronic liver disease, and patients receiving phenytoin or carbamazepine.

Subjects were taken consecutively until the required minimum sample size was met. Subjects' data included name, sex, age, weight, height, NS diagnosis, urea and creatinine levels, blood albumin levels, and serum zinc and serum homocysteine levels. Serum zinc level was measured by ICP-MS method using an Agilent 7700 instrument with required blood specimens of 250-750 μL. Serum homocysteine level was measured by chemiluminescent method using an Advia Centau tool, requiring blood specimens of 100-200 μL.

The normal range of serum zinc levels used in this study was based on age-dependent constraints with the following ranges: age <6 months: 26-141 μg/dL, 6 months to 12 months: 29-131 μg/dL, 1 to 2 years: 31 -201 μg/dL, 2-4 years: 26-116 μg/dL, 4 to 6 years: 48-119 μg/dL, 6 to 10 years: 48-129 μg/dL, 10 to 14 years: 25-148 μg/dL, and 14 to 18 years: 46-130 μg/dL. From the normal serum zinc values, we classified zinc <40 μg/dL as zinc deficiency for children above 4 years old.

With a 5% significance level and 80% power of the test, the coefficient of the relationship between x and y with r -0.55 yielded a minimum required sample size of 20 subjects. Statistical analysis was performed using Pearson's correlation test to determine the correlation between serum zinc and serum homocysteine levels. The type of relationship was determined by double regression analysis. Data analysis was performed using *SPSS version 21 for Windows* software. This study was approved by the Health Research Ethics Commission of Dr. Hasan Sadikin Hospital, Bandung.

Results

From November 2017 to January 2018, 23 study subjects met the inclusion criteria and no children were excluded. There was no significant difference in numbers of boys (48%) and girls (52%). The youngest subject was 24 months (2 years) and the oldest was 200 months (16 years and 8 months). The mean age of subjects was 161 months (13 years and 5 months). Most NS diagnoses were steroid-resistant NS (70%) followed by frequent relapse NS (17%), first attack NS (9%), and steroid-dependent NS (4%) (**Table 1**).

Table 2 describes the statistical analysis of the variables studied. Subjects' mean serum homocysteine level in this study was 10.37 (SD 4.11, range 5.3-19.5) μmol/L. The mean zinc concentration was 51.3 (SD 29.69, range 15-124) μg/dL. The mean blood albumin level was 1.46 (SD 0.99, range 0.3-3.5) g/dL. Shapiro-Wilk data normality test showed that the homocysteine data had a normal distribution (P=0.093).

Table 3 shows the correlations between variables studied. Pearson's correlation showed that serum zinc and serum homocysteine were not linearly correlated (r -0.173, P=0.430). For the linear model, age (months)

had a significant correlation with serum homocysteine levels, with the equation: homocysteine levels = 6.10 + 0.042 x age (months); with R^2=33.1%. This result indicated that 33.1% of variation in homocysteine levels was influenced by age (months)

Figure 1 shows the relationship between serum homocysteine and zinc levels in children with NS. After controlling for age and albumin, the relationship between serum zinc and homocysteine in NS patients was significant in a cubic model with the following equation: homocysteine = -4.572 + 0.735 x zinc - 0.0012 x $zinc^2$ + 0.00005 x $zinc^3$ x age (months) (R^2 multiple=53.2%; P=0.012). This result indicated that 53.2% of homocysteine variation was influenced by serum zinc, and 46.8% by other factors.

The distribution of serum zinc and homocysteine data showed that serum zinc levels below 40 μg/dL would not affect the levels of homocysteine and even tended to increase the serum homocysteine levels. Conversely, when zinc levels were above 40 μg/dL (higher serum zinc levels), serum homocysteine levels tended to be lower.

Table 1. Characteristics of study subjects

Characteristic	(N=23)
Sex, n (%)	
Male	11
Female	12
Age, month	
Mean (SD)	161.6 (56.2)
Range	23-200
Diagnosis, n (%)	
First attack NS	2
Frequent relapse NS	4
Steroid-dependent NS	1
Steroid-resistent NS	16

Discussion

This study provides information on serum homocysteine and zinc levels, as well as the correlation between them in children with NS. Hyperhomocysteinemia (Hhcy) was noted in 52% of our subjects. Similarly, a previous study showed an increase in Hcy levels and decreased vitamin B12 levels in 42 children with NS in Nigeria.[9] Hyperhomocysteinemia in NS patients was suggested to be associated with an inhibition of the homocysteine remethylation process or disruption in cysteine clearance.[9]

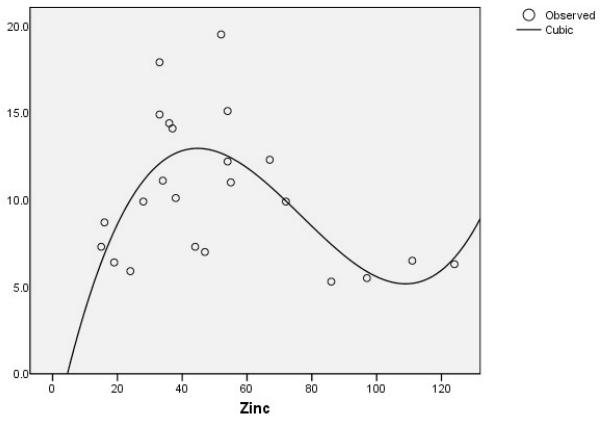

Figure 1. Diagram of the relationship between serum zinc and homocysteine in NS patients

Table 2. Serum zinc, homocysteine, and albumin levels in NS patients

Variables	Mean (SD)	Median (range)	Data normality test (P value*)
Serum homocysteine, μmol/L	10.37 (4.11)	9.90 (5.3-19.5)	0.093
Serum zinc, μg/dL	51.13 (29.69)	44.0 (15-124)	0.024
Albumin, g/dL	1.46 (0.99)	1.1(0.3-3.5)	0.007

Note: * Based on Shapiro-Wilk test, P value >0.05 normal data distribution

Table 3. Correlation between variables studied

Correlation	Correlation coefficient (r)	P value*
Homocysteine and age	0.624	0.001
Homocysteine and zinc	-0.173	0.430
Albumin and homocysteine	0.336	0.117

*based on Pearson's correlation test

The normal homocysteine value in adults is 5-15 μmol/L, but there is no consensus on normal levels in children.[10] Naseri *et al.* in his study on Hhcy in children and young adults on dialysis, defined it in children by age: >8.3 μmol/L for children aged 2-10 years, >10.3 μmol/L for children aged 10-15 years, and >11.3 μmol/L for children aged 15-18 years.[10]

Statistical analysis revealed that age (months) was linearly correlated with homocysteine levels, with the following equation: homocysteine level = 6.10 + 0.042 x age (months), with R^2=33.1%. This equation indicates that 33.1% of homocysteine level variation was influenced by age and was consistent with a study by De Laet *et al.* who observed that total homocysteine concentrations were lowest in younger children and increased with age.[11]

Hyperhomocysteinemia most commonly occurs in steroid-resistant NS. As many as 68% of our patients with steroid-resistant NS had Hhcy. This result was consistent with other clinical studies that showed a pathogenic effect of Hhcy that caused podocyte injury and glomerulosclerosis.[5,12] Hyperhomocysteinemia can cause injury and glomerular sclerosis due to impaired extracellular matrix metabolism, decreased protection from nitric oxide (NO), and increased reactive oxygen species (ROS).[13] Subjects' mean serum zinc level was 51.3 (SD 29.69, range 15-124) μg/dL, with zinc deficiency in 8 of 23 (35%) subjects. Similarly, Dwivedi *et al.* showed a decrease in zinc and copper levels in patients with NS.[14] The serum zinc level decrease in NS was associated with increased urinary zinc excretion through mechanisms of renal secretion or reabsorption. Other mechanisms for reduced zinc include nutritional deficiencies, low intake of zinc in the diet, as well as decreased absorption of zinc or increased secretion of zinc into the intestine.[8]

Zinc deficiency occurs most frequently in frequent relapsie NS patients, followed by steroid-resistant NS. No zinc deficiency was observed in the first attack NS patients. Previous studies by Arun *et al.* and Bhatt et al. showed that zinc supplementation may decrease the incidence of relapse in NS patients,[15,16] due to the effect of zinc in reducing the risk of infection, particularly infection of the gastrointestinal and respiratory tracts.[16] Zinc deficiency causes down-regulation of Th1 cytokines, relative Th-2 bias, and increased risk of infection. Zinc supplementation strengthens IL-1 and interferon gene expression, thereby restoring the Th1 immune response. The balance of Th-1–Th-2 cytokines may prevent the occurrence of relapse in NS.[15]

Homocysteine did not linearly correlate with zinc in our study. After controlling for age and albumin level, homocysteine was observed to have a significant association with zinc by a cubic model. The equation for this model was homocysteine = -4,572+0.735 x zinc – 0.0012 x $zinc^2$ + 0.00005 x $zinc^3$ x age (months). The R^2 coefficient determinant of 53.2% means that 53.2% of the variation in homocysteine was determined by zinc.

Other factors that may affect homocysteine include genetic abnormalities such as homocysteinuria, cystathionine beta synthase (CBS), methylentetrahydrofolate reductase (MTHFR), and Down syndrome. In addition, physiological determinants such as gender, age, kidney function, and muscle mass, as well as lifestyle determinants such as coffee and alcohol consumption, smoking, exercise, as well as certain clinical conditions such as blood folic acid and vitamin B12 levels, hyperproliferative disorders, hypothyroidism, diabetes, and consumption of anti-seizure medicines may affect homocysteine levels.[17]

The distribution of serum homocysteine and zinc levels in this study showed that the zinc < 40 μg/dL had limited association with serum homocysteine levels, and even tended to increase the serum homocysteine. This finding was in agreement with a study on the effects of zinc deficiency and zinc supplementation on homocysteine levels and enzyme-related expression in rats. Jing *et al.* showed that zinc deficiency increased serum homocysteine levels and reduced mRNA levels of methionine synthase enzymes.[7]

Conversely, when zinc levels were higher (above 40 μg/dL), serum homocysteine levels tended to be lower. As such, zinc levels above 40 μg/dL may have a protective value against hyperhomocysteinemia. Previous studies have shown that zinc supplementation may help lower Hcy levels. Heidarian et al. observed that zinc supplementation in patients with type 2 diabetes mellitus with microalbuminuria decreased serum Hcy levels.[18] Jing *et al.* also showed significant negative correlations between serum homocysteine and zinc levels in rat liver and kidneys (r -0.632; P<0.01 and r -0.534; P<0.05, respectively).[7]

A possible pathomechanism to explain such a correlation is the presence of zinc metalloenzymes in Hcy metabolism, two of which are methionine

synthase (MS) and betainehomocysteine methyltransferase (BHMT).[7,17,19] Homocysteine is metabolized from the body via transulfuration and remethylation pathways.[7] In the remethylation path, homocysteine is converted to methionine and requires methylcobalamin as a cofactor and 5-methyltetrahydrofolate as the substrate. This process also requires methionine synthase (MS) to catalyze the methyl transfer of 5-methyltetrahydrofolate from Hcy.[7] Other remethylation pathways are regulated by BHMT, which catalyzes the transfer of methyl from betaine to Hcy to form dimethylglycine and methionine.[7,14,20]

A limitation of this study was that subjects of this study were not examined for genetic disorders such as CBS and MTHFR gene defects that could affect homocysteine levels. We also did not measure folic acid and vitamin B12 levels, which are currently the standard therapy for hyperhomocysteinemia.

In conclusion, homocysteine is not linearly correlated with zinc, but is significantly associated by a cubical model, with a coefficient determinant of R^2=53.2%.

Conflict of Interest

None declared.

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Case Report

Motor clinical progression in a series of pediatric Duchenne and Becker muscular dystrophy cases

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Muscular dystrophy is a neuromuscular disorder that begins with muscle weakness and impaired motor function. Duchenne muscular dystrophy (DMD) is more severe and destructive than Becker muscular dystrophy (BMD), and both are progressive in nature. These 2 types of muscular dystrophy are caused by mutations in related to X-chromosome genes.[1] The mutations that occur in DMD are nonsense mutations. Deletion is present in 60% of DMD cases, while duplication occurs in 10% of DMD cases, resulting in loss of dystrophin protein. Mutations in BMD are missense mutations, so dystrophin is still formed, but in decreased amounts and quality.[2,3]

The prevalence of DMD was reported to be three times greater than that of BMD, with a prevalence of 1.02 per 10,000 male births *vs.* 0.36 per 10,000 male infants, respectiveley.[4] Anatomical pathology examination revealed loss of dystrophin in the examination of muscle biopsy without the presence of evidence leading to other neuromuscular diseases. Clinical DMD symptoms begin to appear at the age of 2-4 years. The child is observed to fall often and has difficulty climbing stairs. Muscle weakness worsens, especially in the upper limbs, continuing with heart and respiratory problems. The main causes of death in DMD are respiratory failure and heart failure.[5] The BMD has varied clinical symptoms, beginning with the appearance of myalgia, muscle cramps, and arm weakness progressing towards myopathy. Some patients are asymptomatic until the age of 15, but 50% of patients show symptoms at age 10, and almost all by age 20.[6] **[Paediatr Indones. 2019;59:104-12; doi: http://dx.doi.org/10.14238/pi59.1.2019.104-12].**

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The DMD diagnosis is based on clinical history, physical examination, muscle biopsy examination, and genetic testing. Muscle biopsy reveals the progression of muscle degeneration and regeneration. Histopathological examination of the muscle biopsy with immunohistochemical staining can show diminished or missing dystrophin.[7] However, one study that examined progression in DMD using MRI to observe muscle cross-sectional area (CSA) at various ages, showed differential fatty-tissue infiltration across

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the lower extremity muscles. The CSA were lower in boys who suffer from older DMD when compared to younger patients.[8]

Clinical motor progression in muscular dystrophy may worsen to the point that patients lose the ability to climb the stairs and have difficulty standing on their own. The progression leads to disability and eventual death. A previous study has shown that motor degeneration in DMD and BMD patients correlated with age.[9] Decreased gross motor function can be used to predict motor stages in DMD patients, but fine motor function does not show a decline.[10] Malnutrition is found in 50% of DMD patients, and if not properly managed, may aggravate the patient's muscular dystrophy condition. Multidisciplinary management is expected to slow the deterioration of this disease and optimize patient quality of life. Here we present six cases of DMD and BMD who were diagnosed with gastrocnemius muscle biopsy, hematoxylin eosin (HE), and immunohistochemical (IHC) staining using dystrophin antibody, followed by a reading of histopathologic preparations. We followed up motor clinical progression in patients who had been diagnosed for more than one year.

The Cases

We used medical record data from six patients who had been diagnosed with DMD or BMD for more than one year, from January 2012 to December 2016 at Dr Sardjito General Hospital, Yogyakarta. Pediatric neurologists evaluated the patients' motor clinical progression, gross motor skill (by motor function measure score/MFM), fine motor skill (by MACS score), nutritional status, steroid therapy use, and physiotherapy undertaken.

The motor clinical stages of DMD was divided into 5 stages, namely (I) Presymptomatic: the diagnosis at this stage is made from an increase in creatine kinase levels or if there is a family history. Children may experience developmental delays but have not had a walking disorder; (II) Early ambulatory: marked by a gower sign, waddling gait, walking with heels, the child can climb up the stairs; (III) Late ambulatory: marked loss of ability to climb stairs and get up from the floor; (IV) Early non ambulatory: children can still maintain body position, limited self movement and begin to experience scoliosis; (V) Late non ambulatory: the function of the upper limb and the ability to maintain posture are very limited.[11] Gross motor function in muscular dystrophy is measured by MFM score that includes 3 motor functions: standing and moving in sub-section (D1), upper limb motor function (D2), and lower limb motor function (D3).[10] The decrease in walking capacity (D1) within a year is 40%, the total decrease in motor function in one year from all aspects is 70%.[12]

The manual ability classification system (MACS) was designed to classify how children with cerebral palsy use their hands when handling objects in daily activities that require upperlimbs. Fine motor function in muscular dystrophy was examined by MACS score that divided into 5 categories: (1) handles objects easily and succesfully; (2) handles most objects but with somewhat reduced quality and/or speed of achievement; (3) handles object with difficulty, needs help to prepare and/or modify activities; (4) handles a limited selection of easily managed objects in adapted situations, requires continuous support and assistance for even partial achievement of the activity; (5) does not handles objects and has severely limited ability to perform even simple actions.[13]

Children's nutritional status was measured by WHO chart 2006. The cut off points used were according to z-score. Children aged less than 5 years were declared severely malnourished (severely wasted) if weight for length/height is below -3. Children aged ≥ 5 years old were declared severely malnourished if body mass index (BMI) is below -3. Mild malnourished (wasted) if weight for length/height or BMI between -3 < z score < -2. Normal nutrition if weight for length/height or BMI between -2 < z score < +1.[14]
Patients was categorized as steroid therapy group if they received prednisone in a dose of 0.5-0.75 mg/kgBW/day with good adherence to the therapy.[15] Physiotherapy was defined as routine if was done 3 times a week with standardized physiotherapy from rehabilitation centre.[16]

The six patients' characteristics are shown in **Table 1.**

Case 1

A boy aged 9 years and 9 months was admitted to our hospital with a complaint of often falling when walking, as observed by his parents. The child also

Table 1. Clinical findings in 6 DMD and BMD patients

Muscular dystrophy	Diagnosis			Follow up						
	Sex	Age	Motor clinical stage	Age	Motor clinical stage	Gross motor function score	Fine motor function score	Nutritional status	Steroid use	Physiotherapy
Case 1	M	9 yr 9 mo	DMD	11 yr 7 mo	IV	29.2%	3	Good	No	No
Case 2	M	9 yr 2 mo	II	12 yr 7 mo	IV	31.3%	3	Severe malnutrition	No	Yes
Case 3	M	7 yr	III	9 yr	III	57.3%	2	Good	Yes	Yes
Case 4	M	8 yr 10 mo	II	13 yr	IV	38.5%	3	Good	No	No
Case 5	M	6 yr 6 mo	II	7 yr 10 mo	II	80.2%	1	Good	Yes	Yes
Case 6	F	16 yr	BMC II III	17 yr	III	41.7%	2	Good	No	Yes

had difficulty climbing stairs and walking on tiptoes. Physical examination showed decreased strength of the lower extremities. His motor clinical stage was II (early ambulatory stage). His enzymes were as follows: serum creatinine kinase (CK) was increased to 9,159 U/L, creatine kinase M-B (CKMB) was 287 U/L, and lactate dehydrogenase (LDH) was 1,936 g/dL. Electroneuromyography (ENMG) showed polymyositis. Hematoxylin eosin staining of a muscle biopsy showed variable muscle fiber size, with fibrosis and infiltration of adipocytes. The IHC staining revealed no evidence of dystrophin protein in the cellular membranes (**Figure 1** and **Figure 2**). Hence, the patient was diagnosed with DMD. At the age of 11 years and 7 months, the patient was reevaluated. Steroid therapy and physiotherapy had not been undertaken. His motor clinical stage had deteriorated to an early non-ambulatory stage (stage IV). The boy's MFM score was 29.2% and MACS score was 3. Upon evaluation, the patient had both impaired gross motor function and fine motor function, but his nutritional status was good.

Case 2

A boy aged 9 years and 2 months was hospitalized with a chief complaint of weakness in the lower extremities for the past year. Gower's sign and Duck sign were observed. From physical examination, he was in late ambulatory motor clinical stage III. Complete blood count was normal. His CK was 10,499 U/L, CKMB 257 U/L, and electrolytes within normal limits. Electroneuromyography (ENMG) revealed myopathy. The HE staining of a muscle biopsy showed adipocyte infiltration in muscle fiber and fibrous tissue replacement. The IHC staining showed no evidence

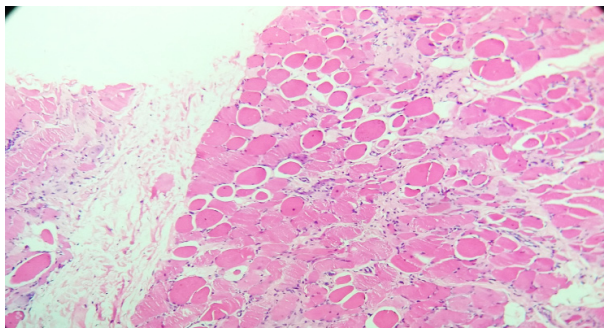

Figure 1. Case 1 HE staining: infiltration of adipocytes in muscle fiber and fibrous tissue replacement

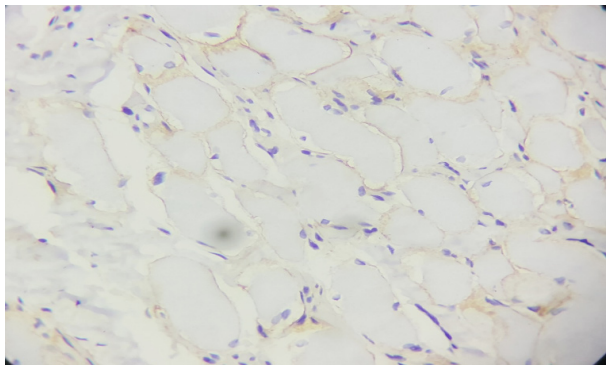

Figure 2. Case 1 IHC staining: no evidence of dystrophin in the cellular membranes

of dystrophin in the cellular membranes (**Figure 3** and **Figure 4**). The patient was diagnosed with DMD. He had never received steroid therapy, but had undergone routine physiotherapy. The patient was reevaluated at the age of 12 years and 7 months. His motor clinical stage was IV and he had impaired gross motor function (MFM score 31.3%) as well as fine motor function (MACS score 3). This patient also suffered from severe malnutrition and scoliosis.

Case 3

A 7-year-old boy was admitted with the chief complaint of frequent falling while walking during the two months prior to admission. He developed weakness in his lower extremities without any history of trauma. He also had difficulty standing from a squatting position and the complaints worsened over time. He had early ambulatory motor clinical stage II. His CK was 10,443 U/L and CKMB was 314 U/L. ENMG revealed myopathy. The HE staining of a muscle biopsy showed adipocyte infiltration in muscle fiber and fibrous tissue replacement. The IHC staining showed dystrophin-negative staining (**Figure 5** and **Figure 6**). He was diagnosed with DMD and entered a longitudinal study for two years. He received both steroid therapy and routine physiotherapy. The patient was reevaluated at 9 years of age. His motor clinical stage had progressed to III and his gross motor function was impaired (MFM score 57.3%). However, his fine motor function was not impaired (MACS score 2). The boy's nutritional status remained good and he was able to attend school using a wheelchair.

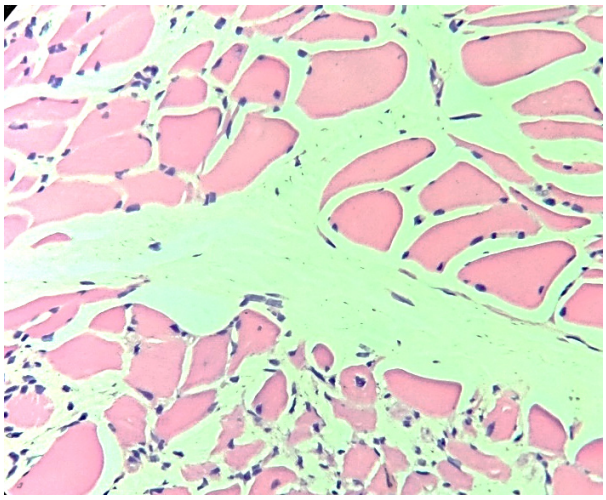

Figure 3. Case 2 HE staining: infiltration of adipocytes in muscle fiber and fibrous tissue replacement

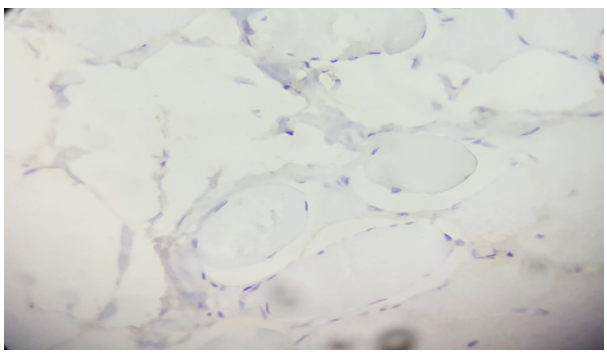

Figure 4. Case 2 IHC staining: no evidence of dystrophin in the cellular membranes

Case 4

A boy aged 8 years and 10 months with a history of difficulty walking for several months was admitted to the hospital for a muscle biopsy. His brother had died after similar complaints. Physical examination showed Gower's sign. This patient was in stage II at diagnosis, but he was able to attend school. His CK was 285 U/L, CKMB 91 U/L, and ENMG revealed

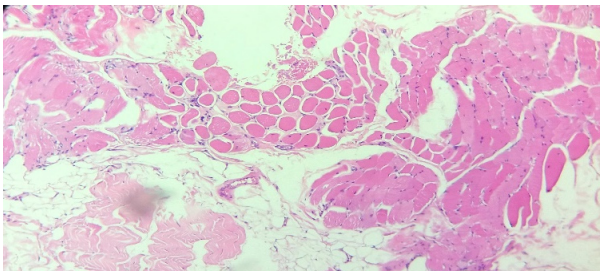

Figure 5. Case 3 HE staining: infiltration of adipocytes in muscle fiber and fibrous tissue replacement

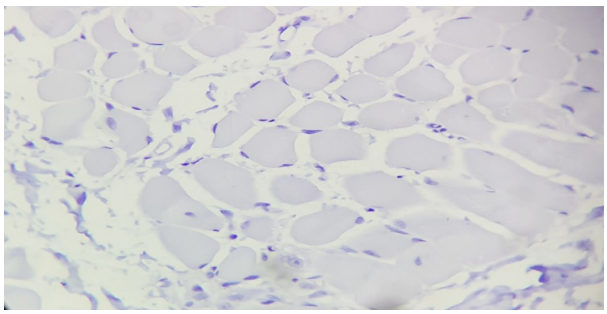

Figure 6. Case 3 IHC staining: no evidence of dystrophin in the cellular membranes

myopathy. The HE and IHC staining led to a diagnosis of DMD due to fibrous replacement in muscle fiber and dystrophin-negative staining (**Figure 7** and **Figure 8**). The patient was evaluated at 13 years of age, at which time his motor clinical stage had progressed to IV and scoliosis was discovered. From history-taking, we found that the family had refused medication and physiotherapy. The boy had impaired gross motor function (MFM score 38.5%) and fine motor function (MACS score 3). The nutritional status of this patient was good at evaluation.

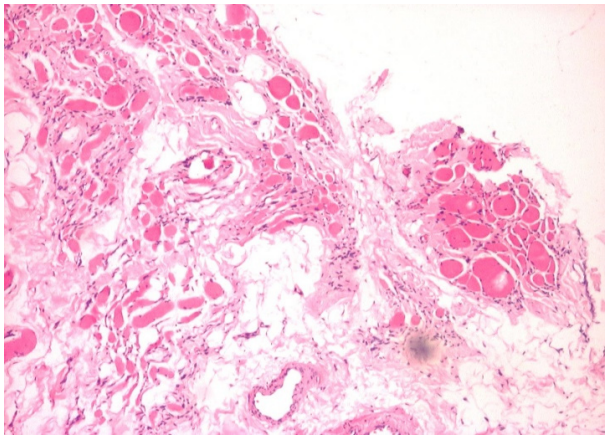

Figure 7. Case 4 HE staining: infiltration of adipocytes in muscle fiber and fibrous tissue replacement

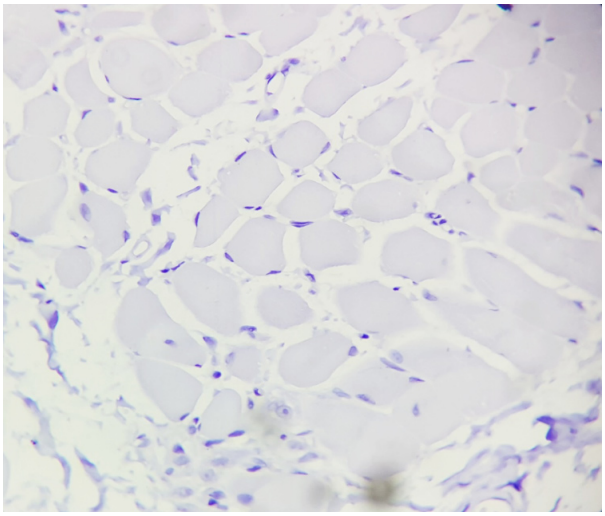

Figure 8. Case 4 IHC staining: no evidence of dystrophin in the cellular membranes

Case 5

A boy aged six years and six months was admitted to our hospital due to frequent falling while walking since six months prior, without a history of trauma. His grandfather's brother reportedly had similar signs. Physical examination revealed stage II motor clinical muscular dystrophy. He had CK 16,550 U/L, CKMB 831 U/L, and his ENMG showed myopathy. The HE staining showed muscle fibers of variable sizes with infiltration adipocytes and fibrosis. The IHC staining showed partial staining of dystrophin in the cellular membranes (**Figure 9** and **Figure 10**). This patient was diagnosed with BMD and was enrolled in a longitudinal study. After one year, the reevaluation showed motor clinical stage II, and no impairment in gross or fine motor function (MFM score 80.2% and MACS score 1). This patient received steroid therapy and routine physiotherapy. His nutritional status was good.

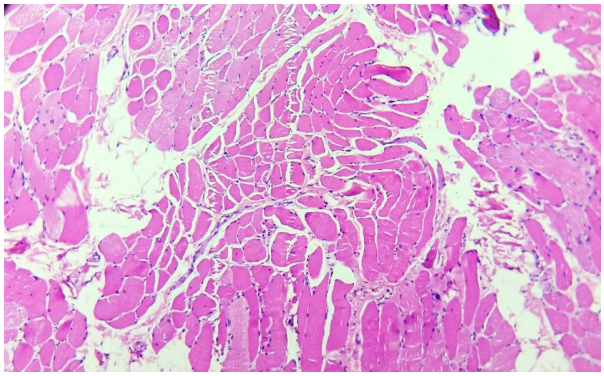

Figure 9. Case 5 HE staining: muscle fibers of variable sizes with infiltration of adipocytes and fibrosis

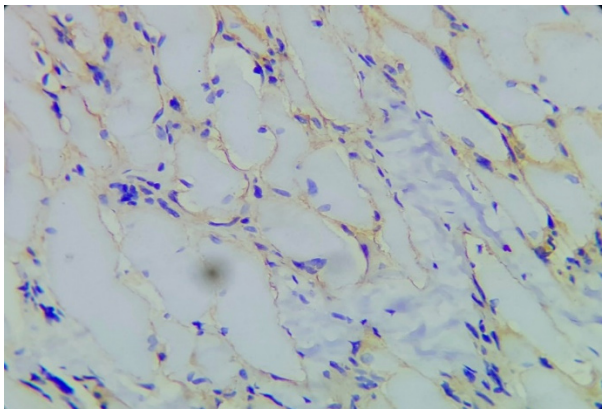

Figure 10. Case 5 IHC staining: evidence of dystrophin in the cellular membranes

Case 6

A girl aged 16 years was admitted because of weakness in her lower extremities and difficulty walking. She had both duck and Gower's signs. Her brother had died at 17 years of age with similar signs that worsened. Her serum CK was 2,412 U/L. The patient had previously undergone ENMG examination, which revealed polyneuropathy. The HE staining of the muscle biopsy showed muscle fibers of variable sizes with infiltration of adipocytes and fibrosis. The IHC staining showed that dystrophin was partially stained, indicating decreased amounts in the cellular membranes (**Figure 11** and **Figure 12**). She was diagnosed with BMD and motor clinical stage of III. She did not receive steroid therapy, but had routine physiotherapy. At the one-year evaluation, her motor clinical stage had not deteriorated. Her gross motor function was impaired (MFM score 41.7%), but her fine motor function was good (MACS score 2). The girl's nutritional status was good and at 17 years of age she was still able to attend school using a wheelchair.

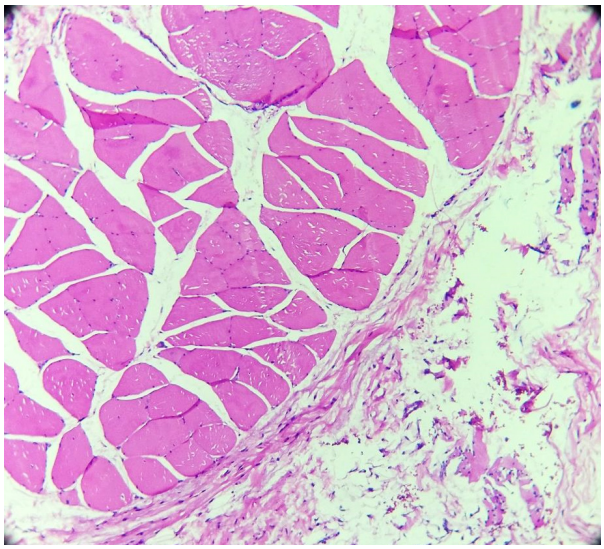

Figure 11. Case 6 HE staining: muscle fibers of variable sizes with infiltration of adipocytes and fibrosis

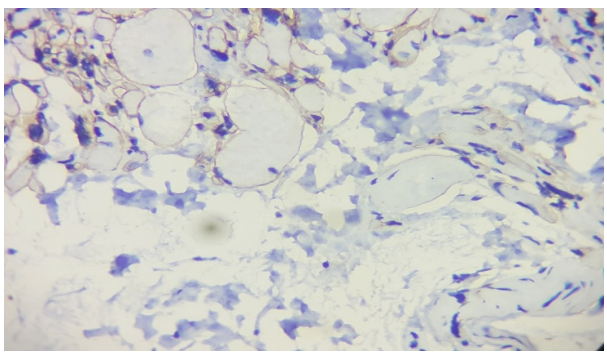

Figure 12. Case 6 IHC staining: evidence of dystrophin in the cellular membranes

Discussion

A Centers for Disease Control and Prevention Muscular Dystrophy Surveillance Tracking and Research Network (MD STARnet) team followed the birth of baby boys from 1982-2011 in the United States. The most common muscular dystrophy was DMD, with a prevalence of 2.83 times more than BMD (1.02/10,000: 0.36/10,000).[4] Currently at Dr. Sardjito General Hospital, Yogyakarta, diagnosis of muscular dystrophy is done by patient history, onset of symptoms, family history of similar disease, serum creatine kinase, and muscle biopsy.

All 6 cases walked had abnormal gait. Scoliosis was found in one patient with motor clinical stage IV. Clinical progression of DMD can be assessed by periodic examinations, including changes in walking, clinical motor weakness, motor capacity examination with a 6-meter walking test, and respiratory function checks. Systematic search results suggest that motor clinical progression in DMD begins at age 8 and becomes more severe by the age of 16-18 years. On average, symptoms start at the age of 10 years. Average motor clinical deterioration occurs at the age of 19.9 years. Scoliosis was found in 3.9% of DMD cases in Japan and 52.1% of cases in France. Loss of motor abilities in 1 year was estimated at about 3%, and 29% in 3 years after the onset of symptoms. One study mentioned a loss of motor skills up to 43% within 1 year of observation.[17]

Muscular dystrophy diagnoses in this case series were obtained through gastrocnemius muscle biopsy HE staining and IHC staining using anti-dystrophin antibody. Histopathologic preparations can be used to determine the degree of infiltration of adipocytes and fibrosis, which indicate the degree of muscle damage. The DMD patients have dystrophin-negative staining in muscle fiber membranes, whereas BMD patients have partial staining of dystrophin proteins.[7] A previous study at Dr. Sardjito General Hospital

reported that a diagnostic test of stage III muscle biopsy with HE staining had 50% sensitivity (95%CI 1 to 99), and 40% specificity (95%CI 15 to 65). The accuracy of histopathologic diagnoses of grade 4 muscle biopsy with HE staining was 50% sensitivity (95%CI 1 to 99) and 60% specificity (95%CI 35 to 85). The IHC examination is considered reliable for DMD diagnosis, but not for BMD diagnosis.[18] A study showed that myofiber examination in muscular dystrophy patients, the anti-dystrophin antibody were lower compared to controls. They also reported that a comparison of various anti-dystrophin antibodies for quantifying purposes revealed degradation of 83% of dystrophin in pediatric DMD patients using ab15277 antibodies, with a dystrophin mean of 4000 intensity values (i.v.). If using MANDYS106 anti-dystrophin antibodies, there was a 70% decrease in dystrophin, with a mean dystrophin yield of 4700 i.v. In contrast, BMD patients had more dystrophin protein, with mean of 13,600 i.v by examination of either ab15277 antibody or MANDYS106.[19]

In mothers with children who have DMD, the risk of her being a carrier was 33%.[20] Another study reported that 51% of DMD patients had a carrier mother and 38% of BMD patients had a carrier mother.[21] A case report on a 14-year-old girl with muscle weakness, was diagnosed with BMD based on chromosome analysis 46, XX, and a deletion in exon 45-55. The BMD is rare in women.[22] The DMD patients aged 9-17 years are often obese due to the effects of steroid therapy. By the age of 17 years, the patient may become malnourished.[23] Malnutrition in such cases has been correlated to muscle function. Increased infiltration of fat tissue in skeletal muscle causes increased fat mass in muscle. Weight loss due to muscle wasting affects physical ability and daily activities of muscular dystrophy patients. Weight loss also occurs due to feeding difficulties, such as swallowing disorders leading to insufficient intake of energy, protein, and micronutrients. The recommended diet for muscular dystrophy patients is 80% of the recommended daily allowance (RDA) in patients who can still walk, and 70% of the total RDA in patients who use wheelchairs.[24]

A study of DMD patients aged 10 to 16 years who underwent prednisone therapy (0.75 mg/kg/day) from diagnosis to an average observation of 8.5 years noted that 40% of patients were still able to stand from a sitting position and 50% were capable of completing a 30-foot running test. Their pulmonary function capacity was also normal. In patients aged 10-13 years, 13% had ventricular systolic dysfunction; in patients > 13 years, 21% had ventricular systolic dysfunction. Only 6% of 16-year-old DMD patients had scoliosis.[25] Our results differed because in Dr Sardjito Hospital the patients did not receive adequate steroid therapy due to poor medication adherence, and delayed diagnosis, as our patients often presented at a later stage of disease.

Physiotherapy can prevent contractures. Recommendations include low intensity exercise and passive stretching. Two contraction mechanisms, concentric and eccentric, are targets for physiotherapy. Concentric contractions occur when the sarcoma is shortened and the muscle cells also shorten because of pressure. Eccentric contractions include elongation of cells and sarcomas under the same emphasis conditions. Eccentric contractions damage the sarcoma and cells, as well as increase inflammation. The target of physiotherapy and activity in muscular dystrophy patients is an increase in muscle cell function.[26] Other studies have shown that physiotherapy significantly prolonged life and decreased disability in muscular dystrophy patients.[27]

Here we show motor clinical progression in six DMD and BMD patients diagnosed from muscle biopsies. The results of a muscle biopsy may be used as a predictive factor of motor clinical progression in muscular dystrophy. Muscular dystrophy should be monitored on an ongoing basis to improve the patient's quality of life.

Conflict of Interest

None declared.

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Original Article

Comparison of GeneXpert MTB to Mycobacterium tuberculosis culture in children with tuberculosis

Betty Agustina, Cissy Kartasasmita, Dany Hilmanto

Abstract

Background Diagnosing tuberculosis (TB) in children is difficult. Typical methods take a long time to achieve results, or have a low sensitivity. GeneXpert is a nucleic acid amplification test used to identify Mycobacterium tuberculosis bacteria (MTB) in only 2 hours.

Objective To compare the sensitivity and specificity of GeneXpert MTB to MTB culture in children with TB, and to assess factors associated with GeneXpert MTB test in predicting which children were likely to have positive results.

Methods This descriptive, analytical study was done in children with suspected TB, aged 1 month to 18 years in Hasan Sadikin Hospital, Bandung, West Java, from January 2016 to December 2017. The data were taken from the medical records and included age, gender, nutritional status, symptoms of TB, chest x-ray, and tuberculin test results. The GeneXpert MTB test was compared to cultures from the same patient, with regards to sensitivity, specificity, and agreement using Kappa index. We analyzed factors associated to GeneXpert MTB test using logistic regression analysis.

Results From 454 inpatients and 1,750 outpatients with suspected TB, there were 251 children who were tested by MTB culture and 722 children tested by GeneXpert MTB. Of the 70 cases who met the inclusion criteria and underwent both tests, factors associated with positive GeneXpert MTB results were age 10 to 18 years, female gender, and positive tuberculin skin test (TST). The GeneXpert MTB test showed sensitivity 78.9% (95%CI 56.7 to 91.5) and specificity 86.3% (95%CI 74.3 to 93.2), with accuracy of 84.3% (95%CI 74 to 91), and agreement value of κ=0.62 (95%CI 41.6 to 82.7).

Conclusion Specificity of GeneXpert MTB is higher than its sensitivity compared to TB cultures in children. The tests were in good agreement. Age 10 to 18 years had the strongest association with positive GeneXpert MTB results. **[Paediatr Indones. 2019;59:113-8; doi: http://dx.doi.org/10.14238/pi59.3.2019.113-8]**.

Keywords: *agreement; GeneXpert; sensitivity; specificity; tuberculosis*

Tuberculosis remains a major problem worldwide. In 2013, 9 million people developed TB and 1.5 million people died from the disease.[1] Every year, TB in children accounts for at least 6% of the global burden of disease. These numbers (530,000-999,792 cases) underestimate the burden of childhood TB, which is higher due to difficulty in diagnosing childhood tuberculosis, emphasizing the need for improved diagnostics.[2]

Tuberculosis in children manifests with severe dissemination and clinical presentations. In this age group, hematogenous and lymphatic spread of the primary infection cause extrapulmonary symptoms such as miliary and meningitic disease. Young children with severe and complicated disease have a much higher mortality rate than adults.[2] Tuberculosis is among the 10 major causes of mortality among children, with a global estimate of 130,000 deaths per year.[3]

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In children, culture methods have a greater, yet highly variable, sensitivity than other typical diagnostic methods. For this reason, microbiological confirmation of childhood tuberculosis is rarely performed, and clinical diagnosis relies on a combination of signs, symptoms, radiological findings, and identification of a tuberculosis contact.[4] Newer tools such as nucleic acid amplification tests have a slightly higher sensitivity of 40-60%. Other diagnostic tests, including the tuberculin skin test and the interferon gamma release assay, are limited in differentiating between latent infection and active disease.[5] There is an urgent need for a rapid, sensitive, and specific test for tuberculosis and for identification of drug-resistant disease in children.[6] The recent innovation of the Xpert MTB/RIF test by *Cephedi* (Sunnyvale, CA, USA) has greatly transformed the field of TB diagnostics. The Xpert MTB/RIF test simultaneously detects Mycobacterium tuberculosis (MTB) and resistance to rifampicin using real-time polymerase chain reaction (PCR) analysis, and produces results within 2 hours. The other main advantage of the Xpert MTB/RIF compared to traditional PCR methods is that it is fully automated.[7]

In the 2011 policy statement on the Xpert MTB/RIF test, the World Health Organisation (WHO) recommended the test as an initial diagnostic tool among children with suspected HIV-associated TB or multi-drug resistant (MDR) TB, based on successful data in adults.[8] There has been limited published data on the utility of the Xpert MTB/RIF test in the pediatric population with tuberculosis. Nicol *et al.* found an overall sensitivity of 100% for smear-positive/culture-positive cases, 61.1% for smear-negative/culture-positive, and specificity of 98.8% when two induced sputum samples were assessed in children aged less than 15 years with suspected pulmonary tuberculosis in Cape Town, South Africa.[6] Statistical guidance on reporting results from studies evaluating diagnostic tests was used to calculate sensitivity and specificity of the assay and value of agreement with Kappa scores.[9] Sekadde *et al.* in Uganda described clinical characteristics associated with a positive Xpert MTB test. The clinical characteristics which were independently associated with a positive Xpert MTB test included age > 5 years, a positive history of TB contact, and a positive tuberculin test.[7]

The aim of this study was to assess the sensitivity, specificity, and agreement of the GeneXpert MTB test to MTB culture as the gold standard for the diagnosis of childhood tuberculosis in Hasan Sadikin Hospital and describe factors associated with positive GeneXpert MTB test results.

Methods

This descriptive, analytical study with cross-sectional design, was done with data collected from medical records of children with TB from January 2016 until December 2017 at Hasan Sadikin Hospital, Bandung, West Java, Indonesia. The study inclusion criteria were inpatients and outpatients in Hasan Sadikin Hospital aged 1 month until 18 years, diagnosed with suspected TB based on the WHO case definition for a TB suspect[10,11] who underwent MTB culture and GeneXpert testing, and had complete medical records. We excluded patients with incomplete medical records. The study forms were filled in based on secondary data from medical records.

All statistical analyses were performed using the *SPSS software (version 15.0)*. Chi-square test was done to analyze for associations with positive GeneXpert test results. Results with P value less than 0.25 were analyzed by multivariate logistic regression; and P values less than 0.05 were considered to be statistically significant. Sensitivity and specificity were calculated for the diagnostic test. Kappa analysis was used to determine agreement between GeneXpert MTB and solid MTB culture as the gold standard. Following conversion, four levels of agreement for Kappa were reported: <0.40 (poor), 0.40-0.59 (fair), 0.60-0.80 (good), and >0.80 (excellent).[12,13] The study was approved by the Research Ethics Committee of Hasan Sadikin General Hospital, Bandung.

Results

During the two-year study period, there were 454 inpatients and 1,750 outpatients with the diagnosis of TB. The laboratory data showed 251 MTB cultures and 722 GeneXpert MTB results were positive. Of these, only 70 children underwent both GeneXpert MTB and MTB culture tests. The general characteristics of

subjects are shown in **Table 1**. The age group with the most TB cases was 10 to 18 years, and most of them were female (61.4%). Of the 70 subjects, most had well-nourished nutritional status (64.3%).

The factors that were associated with positive GeneXpert MTB results are listed in **Table 2**. Factors with P value less than 0.25 were entered into the logistic regression model shown in **Table 3**. From three factors associated with GeneXpert test, age 10 to 18 years had the strongest association.

There were 22 positive GeneXpert results and 19 positive culture results (**Table 4**). Gene Xpert MTB test identified MTB in 15 of 22 case-confirmed cultures, with higher specificity 86.3% (95%CI 74.3 to 93.2) than sensitivity 78.9% (95%CI 56.7 to 91.5%). The value of agreement was measured by Kappa index κ=0.62 (95%CI 41.6 to 82.7), with good agreement.

Discussion

Microbiological confirmation of childhood TB with culture is rarely performed and clinical diagnosis

Table 1. Characteristics of subjects

Characteristics	Total (N=70)
Age, n (%)	
1 month - 1 year	7 (10)
1-4 years	20 (28.6)
5-9 years	19 (27.1)
10-18 years	24 (34.3)
Gender, n (%)	
Male	27 (38.6)
Female	43 (61.4)
Nutritional status, n (%)	
Low	25 (35.7)
Well	45 (64.3)
Signs and symptoms, n (%)	
Cough ≥ 2 weeks	17 (24.3)
Fever ≥ 2 weeks	38 (54.3)
Lymph node enlargement	37 (52.8)
History of contact, n (%)	17 (24.3)
Type of TB, n (%)	
Pulmonary	46 (65.7)
Extrapulmonary	17 (24.3)
Tuberculin skin test, n (%)	
Positive	36 (51.4)
Negative	33 (47.1)
Chest x-ray, n (%)	51 (72.8)

Table 2. Factors associated with positive GeneXpert MTB results

Variables	OR adjusted*	95% CI**	P values
Age (10-18 years old)	13.00	2.9 to 57.92	0.001
Gender (female)	6.42	1.35 to 30.59	0.02
Nutritional status	0.61	0.15 to 2.50	0.49
History of contact	0.43	0.09 to 1.88	0.26
TST (positive)	4.26	1.06 to 16.98	0.04
Lymph node enlargement	3.44	0.72 to 16.48	0.12

*OR=odds ratio;** CI=confidence interval

Table 3. Multivariate logistic regression analysis of factors associated with positive GeneXpert MTB test results

Variables	OR adjusted*	95% CI**	P values
Age (10-18 years old)	8.39	2.32 to 30.39	0.001
Gender (female)	5.15	1.24 to 21.37	0.024
TST (positive)	3.64	1.02 to 12.92	0.05

*OR=odds ratio;** CI=confidence interval

Table 4. Sensitivity, specificity, accuracy, and Kappa index of Gene Xpert MTB compared to culture tests

Gene Xpert	Culture		Sensitivity (95%CI)	Specificity (95%CI)	Accuracy (95%CI)	Kappa index (95%CI)	P value
	Positive	Negative					
Positive	15	7	78.9 (56.7 to 91.5)	86.3 (74.3 to 93.2)	84.3 (74 to 91)	62.1 (41.6 to 82.7)	0.549
Negative	4	44					

depends on a combination of signs, symptoms, radiological results, and identification of TB contact.[4] Culture is the gold standard for microbiological confirmation, but it takes 2 to 8 weeks to yield results.[14] In our study, we compared GeneXpert MTB test to cultures as the gold standard to detect MTB from respiratory specimens. There were 251 children who underwent MTB culture and 722 children who underwent GeneXpert MTB testing according to Clinical Pathology Laboratory and MDR TB outpatient data. However, only 70 children underwent both tests. The proportion of positive GeneXpert test (31.4%) was higher than in a previous South African study.[6] A negative GeneXpert test result did not necessarily exclude a TB diagnosis, given the fact that the test was unable to identify 27% of children with culture-confirmed tuberculosis. A clinical decision in the context of the patient, therefore, is important in initiating anti-tuberculosis therapy even for children with negative GeneXpert MTB test results.[7]

The GeneXpert MTB assay performance has been previously evaluated mostly on sputum samples collected from adult TB patients, showing a high sensitivity in smear and culture-positive specimens (98-100%), but a much lower sensitivity in smear-negative sputum specimens (43-70%).[15,16] Few such studies have been conducted in children. Nicol et al. showed an incremental increase in sensitivity of 27.8% for GeneXpert MTB, with specificity of 98.8%.6 Bunyasi et al. showed low sensitivity (26.7% for induced sputum samples and 22.6% for gastric lavage samples) and high specificity (100% for induced sputum samples and 99.6% for gastric lavage samples).[17] Our study showed that the GeneXpert MTB assay had 78.9% sensitivity (95%CI 56.7 to 91.5) and 86.3% specificity (95%CI 74.3 to 93.2). The Kappa value was κ=0.62, indicating a good level of agreement.[12,13]

Other studies also reported good agreement between GeneXpert MTB and cultures. Tang et al. showed a Kappa value of 0.73, in their study of both adults and children.[18] Hasan *et al.* showed very good agreement (Kappa values >0.8), but with stool specimens,[19] and Li *et al.* showed a Kappa value of 0.6 for extrapulmonary TB.[20]

We assessed what factors were associated with GeneXpert MTB test in predicting which children were likely to have positive results. Age (10 to 18 years), female sex, and positive tuberculin skin test (TST) were associated with positive GeneXpert MTB results. Primary infection during adolescence was associated with a high risk of developing adult-type disease. Adult-type disease results from primary infection, endogenous reactivation, or exogenous reinfection. Adult-type disease was most common after recent primary infection in children over 10 years of age.[21] Sekadde *et al.* reported that age >5 years, positive TST, and positive history of TB contact were independently associated with positive Xpert MTB/RIF test results.[7]

Teenagers, especially girls in menarche period, have the highest risk of having tuberculosis after the primary infection during adolescence.[21] Marais et al. showed that adolescent girs were at higher risk of developing tuberculosis after recent primary infection than were boys.[22] Also, unlike younger children who get paucibacillary primary disease, older children, especially those above 10 years of age, are more likely to get reactivation/cavitatory disease, thereby increasing the likelihood of a positive GeneXpert MTB test. Moreover, a positive TST response is a marker of TB exposure and increases the likelihood of TB in a child with suspected pulmonary TB.[7]

This study had some limitations. The data were taken from medical records, but some medical records were incomplete, so they were excluded. Also, this study was conducted only at a 3rd level referral hospital, so we could not generalize to lower service level conditions.

In conclusion, the specificity (86.3%) of GeneXpert MTB is higher than the sensitivity (78.9%), compared to TB culture results in children. GeneXpert MTB and cultures had good accuracy and agreement. The factor most strongly correlated to positive GeneXpert MTB results is age 10 to 18 years.

Conflict of Interest

None declared.

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Original Article

Serum eosinophilic cationic protein level and hematological parameters in infants with cow's milk protein allergy

Erkan Dogan, Eylem Sevinc

Abstract

Background Various biomarkers have been investigated in the diagnosis of cow's milk protein allergy (CMPA) in infants. To our knowledge, no prior studies have evaluated serum eosinophil cationic protein (sECP), neutrophil-lymphocyte ratio (NLR), and mean platelet volume (MPV) concurrently in infants with CMPA.

Objective To compare sECP levels, MPV, and NLR in infants with and without CMPA, as well as to investigate the suitability of these parameters as biomarkers in the diagnosis of CMPA.

Methods Fifty-six children with CMPA were compared to 40 healthy, similar to distribution of age and sex normal infants as controls. The serum ECP levels were detected by a chemiluminescence assay. The MPV values were calculated by devices in hemogram parameters. The NLR values were obtained by dividing the neutrophil count by the lymphocyte count.

Results The median sECP level in the CMPA group was significantly higher than in the control group (23.5 and 9.27 ng/mL, respectively; P=0.001). However, there were no significant differences between groups with regards to median MPV (8.5 and 8.6 fL, respectively; P=0.149) and median NLR (0.35 and 0.37 respectively; P=0.637). Correlation analysis of sECP level with MPV and NLR in the CMPA group revealed no significant relationships (P>0.05 for both). In the Receiver-operating characteristic (ROC) curve analysis, the optimal cut-off levels to identify CMPA for sECP, MPV, and NLR were 18.4 ng/mL (60.7% sensitivity, 97.5% specificity, and AUC: 0.831), 10.05 fL (54% sensitivity, 77.5% specificity, and AUC: 0.413) and 0.97 (14.3% sensitivity, 50% specificity, and AUC: 0.528), respectively.

Conclusions The sECP level and blood eosinophil count are significantly higher in infants with CMPA, but MPV and NLR do not differ between infants with and without CMPA. There are also no significant correlations in the CMPA group between sECP and MPV, as well as sECP and NLR. Serum ECP might be useful as a potential biomarker for diagnosing CMPA. **[Paediatr Indones. 2019;59:119-24; doi: http://dx.doi.org/10.14238/pi59.3.2019.119-24]**.

Keywords: *neutrophil/lymphocyte ratio; mean platelet volume; eosinophilic cationic protein; biomarker; cow's milk protein allergy*

Cow's milk protein allergy (CMPA) is the most common cause of food allergies in infancy and is characterized by an inflammatory reaction to milk proteins.[1] Although the incidence of CMPA has increased worldwide, CMPA pathogenesis is not entirely clear. T regulatory cells, antigen-specific T cells, and some mediators secreted by T and B lymphocytes, play roles in CMPA pathogenesis.[2] Although detailed medical

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history, careful physical examination, diagnostic elimination diets, skin prick tests, and specific IgE measurements are helpful for evaluating CMPA, accurate diagnosis remains difficult. This has led to the development of new diagnostic tests.[3]

Eosinophil cationic protein (ECP) is a cytotoxic protein released from eosinophils upon activation. It is often elevated in some allergic diseases, such as asthma, atopic eczema, food protein-induced allergic proctocolitis (FPIAP), and CMPA. As ECP exists in various body fluids, such as serum, saliva, and feces, the measurement of ECP levels can be used as a non-invasive indicator to detect active inflammation in the body.[4,5] In addition to ECP, neutrophil-lymphocyte ratio (NLR) and mean platelet volume (MPV), which can be easily measured in routine tests, have been reported to be potential diagnostic biomarkers for some inflammatory disorders.[6]

The aim of our study was to investigate the sECP levels, MPV, and NLR in infants with and without CMPA and to determine the suitability of these parameters as biomarkers to diagnose CMPA. To our knowledge, to date, these parameters have not been simultaneously evaluated in infants with CMPA.

Methods

This cross-sectional study was carried out at the Department of Pediatric Gastroenterology of Karabuk University Medical Faculty in Karabuk, Turkey, from December 2017 to December 2018. Fifty-six infants with CMPA aged 1.5-11 months and forty healthy infants (similar to distribution of age and sex) were included in the study. The CMPA diagnosis was done according to the *European Society for Paediatric Gastroenterology Hepatology and Nutrition* (ESPGHAN) Guideline: Diagnosis and Management of CMPA.[7] Children with genetic, metabolic, hematological, or infectious diseases were excluded from the study. The study was approved by the Ethics Committee for Non-invasive Clinical Research of Karabuk Training and Education Hospital. Subjects' parents provided written informed consent.

For the whole blood count, blood was collected in 2 mL, ethylenediaminetetraaceticacid (EDTA) tubes and analyzed within 2 hours using a Beckman Coulter LH 780 analyzer. To measure ECP levels, blood was collected in 3 mL glass tubes and tested by a chemiluminescence method using an *Immulite 2000 XPi analyzer Immunoassay System* (Germany).

The data were analyzed with *SPSS version 16.0* software for *Windows*. Results are expressed as mean (SD) or median (range). Shapiro-Wilk test was carried out to determine the normality of data distribution. Values of sECP, white blood cell count, absolute neutrophil count, absolute lymphocyte count, NLR, and MPV had abnormal data distribution, by Shapiro-Wilk test ($P<0.05$), therefore, median values (interquartile range) between groups were determined and compared using Mann-Whitney U test. For age, absolute platelet counts were determined and compared using independent T-test, because of normal data distribution between groups ($P>0.05$). The ROC curve were constructed for the sECP, MPV and NLR. The areas under the ROC curves with 95 % CIs were calculated and compared with each other. Optimal cutoff values for sECP, MPV and NLR, used to discriminate between infants with and without CMPA, were calculated by ROC curves. Sensitivity and specificity of the cutoff values were analyzed. Correlation analyses were evaluated with Spearman's correlation test. A P value of less than 0.05 was considered to be statistically significant.

Results

The mean age of 56 infants with CMPA (32 males, 57%) and 40 controls (23 males, 55%) were 5.30 (SD 1.67) and 5.15 (SD 1.99) months, respectively. There were no statistically significance differences between the two groups with respect to age or gender (**Table 1**).

The median sECP level in the CMPA group was significantly higher than that in the control group (23.5 and 9.27 ng/mL, respectively; $P=0.001$) (**Figure 1**). However, the median MPV levels (8.5 and 8.6 fl, respectively; $P=0.149$) were not significantly different between groups, nor were the median NLRs (0.35 and 0.37, respectively; $P=0.637$) (**Figure 2**). We also noted that the median eosinophil count (320 and 18 mm3, respectively; $P=0.001$) was significantly higher in the CMPA group than in the

Table 1. Comparison of socio-demographic and laboratory characteristics of the CMPA and control groups

	CMPA group (n= 56)	Control group (n=40)	P value
Mean age (SD), months	5.30 (1.67)	5.15 (1.99)	>0.05[a]
Males, n (%)	32 (57)	23 (55)	>0.05
Median sECP (P25-P75), ng/mL	23.5 (4.74-155)	9.27 (3-19)	0.001[b]
Median white blood cell count (P25-P75), ×103/µL	9.01 (6.15-16.65)	9.88 (3.66-14.23)	0.233[b]
Median absolute neutrophil count (P25-P75), ×103/µL	2.17 (1.05-11.49)	2.42 (1.06-5.38)	0.879[b]
Median absolute lymphocyte count (P25-P75), ×103/µL	5.96 (1.1-10.39)	6.14 (2.19-10.71)	0.146[b]
Median eosinophil count (P25-P75), /mm3	320 (3-1770)	18 (12-46)	0.001[b]
Median NLR (P25-P75)	0.35 (0.16-9.5)	0.37 (0.19-1.5)	0.637[b]
Platelet count (SD), ×103/µL	289 (49)	293 (57)	0.712[a]
Median MPV (P25-P75), fl	8.5 (7-10.1)	8.6 (7.6-11.1)	0.149[b]

[a]Independent sample T-test; [b]Mann-Whitney U test

control group. There were no statistically significant differences between the two groups with respect to white blood cell counts, neutrophils, lymphocytes, and platelet counts (**Table 1**). In the CMPA group, correlation analysis of sECP levels with MPV and NLR revealed no significant relationships (P>0.05), but the sECP level was positively correlated to eosinophil count (r=0.666; P=0.001) in the CMPA group (**Table 2**).

The ROC curve analysis was carried out to determine the diagnostic value of sECP and other markers in differentiating between infants with and without CMPA. The optimal cut-off levels for sECP, MPV, and NLR were 18.4 ng/mL (sensitivity 60.7%, specificity 97.5%, and AUC: 0.831), 10.05 fL (sensitivity 54%, specificity 77.5%, and AUC: 0.413),

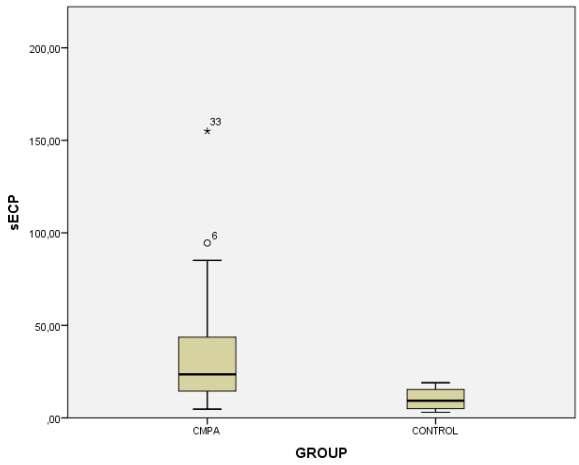

Figure 1. Comparison of median sECP levels in the CMPA and control groups

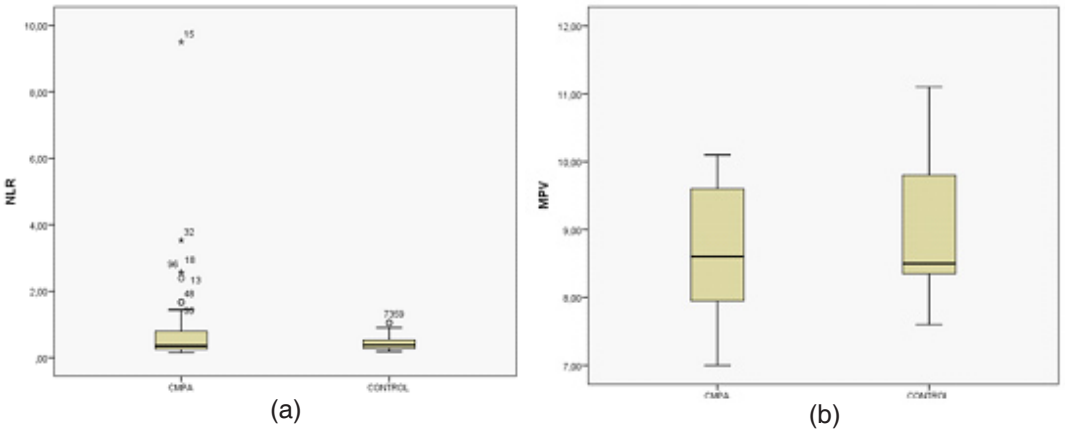

Figure 2. Comparison of median (a) NLR and (b) MPV values in the CMPA and control groups

Table 2. Correlation of sECP with NLR, MPV, and eosinophil count

Parameters	r Value[a]	P value
NLR	0.218	0.17
MPV, fl	0.638	0.64
Eosinophil, mm^3	0.666	0.001

[a]Spearman's rank correlation

and 0.97 (sensitivity 14.3%, specificity 50%, and AUC: 0.528) respectively (**Table 3**). The area under the ROC curve of sECP was statistically significantly higher than the MPV and NLR variables (P=0.001, P=0.637, P=0.149, respectively) (**Figure 3**).

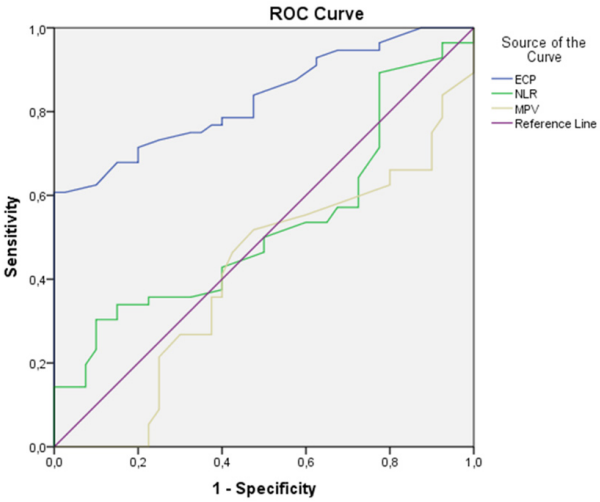

Figure 3. Comparison of ROC curves of ECP, MPV, and NLR.

Discussion

In our study, infants with CMPA had higher sECP levels than controls. On the other hand, there were no significant differences in MPV or NLR values between groups. Moreover, no correlations were observed between sECP level and MPV or NLR values in infants with CMPA.

Eosinophils have large cytoplasmic granules containing protein, such as eosinophil protein X, major basic protein, eosinophil-derived neurotoxin, and ECP.[8] The ECP level and numbers of circulating eosinophils can be increased in several allergic disorders like asthma, atopic dermatitis, and inflammatory diseases. Hence, measurement of ECP is used extensively as an indicator in allergic and inflammatory diseases.[9]

Suomalainen et al. reported that in 5.8 to 43.0-month-old children with CMPA, sECP levels were significantly higher after an oral cow's milk challenge.[10] Consistent with these results, Hidvégi et al. reported that the basic sECP level in children with CMPA (12.4 μg/L) was statistically higher than in the controls (7 μmol/L).[11] Saarinen *et al.* conducted a study in 239 infants with CMPA and reported significantly elevated sECP levels (≥20μg/L) in 35 (15%) infants.[12] Consistent with previous studies, we found significantly higher sECP levels in infants with cows' milk protein induced proctocolitis (CMPIP) compared to controls (23.5 and 9.27 ng/mL, respectively; P=0.001).

The MPV level has been associated with the intensity of inflammation and acts as an acute phase reactant. The severity and duration of inflammation may induce rapid (minutes-hours) shifts in MPV levels.[13] Low MPV levels are seen to represent enhanced consumption of large platelets in inflammatory states like rheumatoid arthritis and familial Mediterranean fever. High MPV levels are associated with various cardiovascular diseases like coronary artery disease, hypertension, and stroke.[14] Recently, conflicting results have been reported on the reliability of MPV as an inflammatory marker for allergic diseases.[15,16] Akelma *et al.* conducted a study in 40 children with chronic urticaria (CU). They reported that the MPV levels of children with CU [7.42 (SD 0.77) fL] were significantly lower than that of controls [7.89 (SD 0.65) fL].17 Consistently, Topal et al. noted that in 6 to 18-year-old children

Table 3. Accuracy and ROC analyses of sECP and other biomarkers to differentiate between infants with and without CMPA

Parameters	Cut-off	AUC	Sensitivity (%)	Specificity (%)	95%CI	P value
sECP	18.4	0.831	60.7	97.5	0.751 to 0.910	0.001
MPV	10.05	0.413	54.0	77.5	0.297 to 0.530	0.637
NLR	0.97	0.528	14.3	50.0	0.411 to 0.645	0.149

with allergic rhinitis (AR), MPV levels were lower than that of controls (7 and 7.6 fL, respectively; P<0.001).[18] On the other hand, Nacaroglu et al. investigated MPV levels in asthmatic children and found no significant differences between groups [8.1 (SD 0.8) fl and 8.2 (SD 0.9) fl, respectively].[19] Likewise, Nacaroğlu et al. reported that MPV levels were significantly higher in children with food protein-induced allergic proctocolitis (FPIAP) than in controls [6.87 (SD 1.3) fl and 8.29 (SD 1) fl, respectively].[20] In contrast, we found no significant difference in MPV levels of infants with and without CMPA (8.5 and 8.6 fL, respectively; P=0.149). All the above studies including ours suggest that alterations of MPV levels may be easily affected by the type and severity of inflammation.

Neutrophil-to-lymphocyte ratio (NLR) is another parameter used to evaluate inflammatory status.7 Despite numerous studies conducted in adults, few studies have evaluated NLR in childhood.[21,22] Moreover, most pediatric studies have focused on NLR in asthma.[23,24] Like studies measuring MPV levels, different results have been reported on NLR in asthma. Zhang et al. observed that NLR was not altered in eosinophilic asthma but increased in neutrophilic asthma.[25] In our study, there was no significant difference in NLR between infants with CMPA and controls (0.35 and 0.37, respectively; P=0.637). Consistent with our findings, Nacaroglu et al. reported no significant differences in NLR values of children with and without FPIAP [0.61 (SD 0.78) and 0.63 (SD 0.87), respectively; P=0.883].20

As mentioned above, various markers have been investigated in the diagnosis of inflammation-related disease. In our study, the area under the ROC curve of sECP performed significantly better than did NLR or MPV (AUC: 0.831, P=0.001; AUC: 0.528, P=0.149; and AUC: 0.413, P=0.637, respectively). Moreover, ECP showed moderate sensitivity and high specificity for diagnosing CMPA (60.7% sensitivity, 97.5% specificity). On the other hand, NLR and MPV showed lower sensitivity and specificity for the diagnosing CMPA (14.3% sensitivity, 50% specificity and 54% sensitivity, 77.5% specificity, respectively).

There were some limitations in this study. As there have been relatively few studies evaluating NLR, MPV and sECP levels in CMPA, we compared our findings to only a small number of studies. Also, other inflammatory markers such as fecal ECP and calprotectin were not evaluated due to funding issues.

In conclusion, infants with CMPA have significantly higher sECP levels than control infants, but NLR and MPV levels do not differ between the two groups. Also, the diagnostic performance of ECP is found to be higher than that of MPV and NLR. Even though NLR and MPV have recently been the source of inspiration as biomarkers for some clinical trials, the ECP might be considered as a potential biomarker for diagnosing CMPA.

Conflict of Interest

None declared.

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Original Article

Serum S100B and intelligence in children with Down syndrome

Nurul Noviarisa, Eva Chundrayetti, Gustina Lubis

Abstract

Background Down syndrome is characterized by physical and mental retardation and caused by chromosome 21 (Hsa21) abnormalities. The S100B is a protein that is overproduced in Down syndrome due to overexpression of chromosome 21 genes. Comorbidities caused by S100B in Down syndrome are cognitive deterioration and early onset of dementia.

Objective To assess for a possible association between S100B protein and intelligence levels in children with Down syndrome.

Method This cross-sectional study included students in a special needs school in Padang, West Sumatera, who had the characteristic clinical features of Down syndrome and trisomy 21 by chromosome analysis. Examination of S100B levels was carried out using an enzyme-linked immunosorbent assay (ELISA) method. Intelligence quotient (IQ) was measured using the 4th edition of the Wechlser Intelligence Scale for Children (WISC-IV) method.

Results A total of 39 children with Down syndrome participated in the study. There were 25 children with mild mental retardation and 15 children with moderate-severe mental retardation. The mean S100B levels were not significantly different between groups [479.1 (SD 204) pg/mL in the mild mental retardation group and 458.7 (SD 158) pg/mL in the moderate-severe mental retardation group; $P > 0.05$]. The mean S100B level was significantly higher in subjects aged ≤ 10 years than in those aged > 10 years [566.9 (SD 210.0) pg/mL and 434.4 (SD 167.2) pg/mL, respectively ($P<0.05$)].

Conclusion There is no association between S100B and intelligence levels in children with Down syndrome. There is a significant association between higher S100B levels and younger age in children with Down syndrome. **[Paediatr Indones. 2019;59:125-9; doi: http://dx.doi.org/10.14238/pi59.3.2019.125-9]**.

Keywords: *Down syndrome; S100B; intelligence level*

Children with Down syndrome have abnormal physical and mental development. Down syndrome is caused by failure of chromosome 21 (Hsa21) to separate during meiosis. This syndrome is characterized by mild-to-moderate mental retardation, craniofacial abnormalities, cardiovascular and gastrointestinal disorders, as well as immune deficiency.[1-4] Down syndrome is one of the most common congenital disorders in children, estimated to comprise 0.45% of human conceptions.[5] The incidence of trisomy Hsa21 worldwide was estimated to occur in 1 per 319-1,000 live births.[6] Idris *et al.* reported that 1,987 Down syndrome patients underwent chromosome analysis at the University of Indonesia from 1992-2004.[7] At Dr. M. Djamil Hospital in Padang, West Sumatera, 95 cases of Down syndrome were reported from 2009 to 2012, but only a small percentage had chromosomal examinations.[8] Chundrayetti noted that 39 children had Down syndrome in special needs schools in Padang in 2017, with chromosome examination results consistent with trisomy 21.[8]

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Down syndrome is the most common cause of intellectual disorders in children. Although the level of cognitive impairment varies widely, 80% of children with Down syndrome have mild-to-moderate intellectual disorders.[1] Overexpression of genes in the Down Syndrome Critical Region (DSCR) on the 21q22.1-21q22.3 segment is thought to be the cause of Down syndrome's clinical manifestations. Some genes in DSCR encode for proteins associated with neurocognitive disorders including amyloid precursor protein (APP), superoxide dismutase 1 (SOD1), dual-specificity tyrosine-phosphorylated and regulated kinase 1A (DYRK1), as well as S100B.[9]

The S100B protein is a member of the S100 protein family, with its gene located on chromosome 21q22.3. In normal conditions, S100B provides protective and neurotropic effects during brain development, the early stages of brain injury, and the regeneration process of injured peripheral nerves. In healthy individuals, S100B levels are at the highest concentration at early age of development, decreasing through adolescence and adulthood, then increasing with aging.[9] At high levels above the normal range, S100B has toxic effects on neurons through excessive production of reactive oxygen species (ROS), which eventually causes neuronal cell and astrocyte apoptosis.[10] The involvement of chromosome 21 in intellectual disorders in Down syndrome is thought to be related to S100B protein. Overexpression of genes on chromosome 21 which occurs in Down syndrome causes an increase of S100B protein levels, leading to neurotoxic effects on neuron cells and astrocytes.[11]

This study was aimed to assess for a possible association between S100B protein and intelligence levels in children with Down syndrome.

Methods

This cross-sectional study was conducted at special needs schools in Padang, West Sumatera, and the Biomedical Laboratory of Andalas University Faculty of Medicine in February to March 2018. This study was approved by the Ethics Committee of the Universitas Andalas Medical School.

The study population comprised students from the special needs schools in Padang who met the inclusion criteria of clinical signs of Down syndrome and trisomy 21 by chromosome analysis. Exclusion criteria were a history of brain infection, brain trauma, epilepsy, cerebral palsy, history of heart surgery, or schizophrenia. Serum S100B was measured using enzyme-linked immunosorbent assay (*Elabscience Biotechnology Co. Ltd*) . The IQ level was measured using the *Wechsler Intelligence Scale for Children* (WISC-IV) 4th edition.[12] Data were processed using *SPSS version 15* software. T-test was used to analyze for an association between serum S100B and intelligence levels. Results with P values <0.05 were considered to be statistically significant.

Results

Demographic characteristics of the 39 children with Down syndrome who fulfilled the inclusion criteria are shown in **Table 1**. Most subjects were male and > 10 years of age. Most subjects' mothers were >35 years of age at the time of childbirth. There were more subjects with mild than moderate-severe mental retardation.

Table 1. Demographic characteristics of subjects

Characteristics	(N=39)
Sex, n	
Male	25
Female	14
Age, n	
≤ 10 years	11
> 10 years	28
Maternal age at childbirth, n	
< 35 years	10
≥ 35 years	29
Intelligence level, n	
Mild mental retardation	25
Moderate-severe mental retardation	14I

The S100B levels in children with Down syndrome were normally distributed (P<0.05), with means of 479.1 (SD 204) pg/mL in the mild mental retardation group and 458.7 (SD 158) pg/mL in the moderate-severe mental retardation group. There was no statistically significant association between mean S100B levels and intelligence in children with Down syndrome (P=0.749) (**Table 2**).

Association analysis between serum S100B level and age is shown in **Table 3**. The mean S100B level was significantly higher in subjects aged ≤ 10 years

Table 2. Analysis of S100B and intelligence levels in children with Down syndrome

Age	S100B level, pg/mL		P value
	n	Mean (SD)	
Mild mental retardation	25	479.1 (204)	0.79*
Moderate-severe mental retardation	14	458.7 (158)	

*T-test

Table 3. Association between S100B level and age in children with Down syndrome

Age	S100B level, pg/mL		P value
	n	Mean (SD)	
≤ 10 years	11	566.9 (210.0)	0.045*
>10 years	28	434.4 (167.2)	

*T-test

than in those aged > 10 years [566.9 (SD 210.0) pg/mL and 434.4 (SD 167.2) pg/mL, respectively (P<0.05)].

Discussion

The majority of study subjects were male, with a male: female ratio of 1.8:1. Johnson in 2006 also found more males with Down syndrome than females.[13] Most Down syndrome children (about 85%) have mild levels of mental retardation, whereas severe mental retardation occurs in 0.3-0.5% of the Down syndrome pediatric population.[14] In our study, mild mental retardation was more common (64.1%) than moderate-severe mental retardation. The incidence of Down syndrome increases in women who give birth at >35 years of age.[7] We also noted that 74.4% of our subjects' mothers were over 35 years of age at the time of childbirth.

Overexpression of S100B in neural progenitor cell (NPC) were isolated from frontal cortex of postmortem fetuses with Down syndrome causes an increase in the formation of reactive oxygen species (ROS) and activation of the stress response. Activation of this pathway results in compensatory aquoporin-4 expression. Aquoporin-4 expression can be induced by direct exposure to ROS, and an inhibition of aquoporin-4 by siRNA resulted in elevated levels of ROS following S100B exposure. Finally, increased levels of ROS induced by S100B and loss of expression of aquoporin-4 led to increase neuronal cell death.[15] Some studies reported increased S100B levels in Down syndrome. Kato *et al.* conducted a study of S100B protein levels in individuals with Down syndrome aged 10-40 years in Japan. They noted higher S100B protein levels in those with Down syndrome compared to individuals without Down syndrome. This high S100B protein level was thought to be related to cognitive impairment in Down syndrome.[16] Similarly, Netto *et al.* showed increased S100B protein levels in 48 children with Down syndrome compared to controls, as well as a possible relationship between S100B protein levels and neurodegenerative lesions that occur in Down syndrome.[9] Previously, Netto et al. had reported higher levels of S100B in amniotic fluid of mothers carrying fetuses with Down syndrome compared to that of mothers carrying normal fetuses.[17]

In our study, the mean S100B level was 479.1 (SD 204) pg/mL in the mild mental retardation group and 458.7 (SD 158) pg/mL in the moderate-severe mental retardation group. Similar to previous studies, the mean S100B level of children with Down syndrome in our study was higher than the normal S100B cut-off value of 20-150 pg/mL. Boussard et al. noted that S100B protein concentration above the cut-off value of 150 pg/mL was considered pathological.[18] S100B protein is produced mainly by astrocytes in the brain, with increasing levels consistently indicative of a neuropathological process. The main advantage of measuring S100B in serum is that increases in serum can be easily measured, providing a sensitive method for detecting central nervous system dysfunction. Although S100B is also produced by other extracranial cells, in previous studies extracranial S100B did not affect serum S100B level.[19]

Many studies support an important role of S100B in central nervous system development.[18,20-24] Extracellular S100B at nanomolar concentrations acts as a potent neurotropic and gliotropic agent. The effects of S100B on cognitive function include increased cell function, suppression of neurovascular inflammation, as well as increased conduction and transmission of nerve impulses.[21] S100B is a potential marker of trauma, infection, or pathological disorders in the central nervous system. In this context, S100B is described as an acute-phase response protein. Increased S100B level in response to

various stressors can lead to pathological symptoms of cognitive impairment. The S100B expression also increases selectively in astrocytes in the aging process, Alzheimer's disease, and in individuals with chromosome 21q22.3 excess. This finding was seen in the pathophysiology of neurodegenerative disorders that are typical of Alzheimer's disease and Down syndrome.[22] Several studies have shown associations between S100B level and cognitive impairment in several disease conditions. Pedersen *et al*. found a significant association between S100B levels and memory disorders in schizophrenic patients.[23] Zhai *et al*. also found a significant association between S100B gene polymorphism and increased level of S100B protein with visuospatial disability in schizophrenic patients.[24] Furthermore, Li *et al.* reported increased S100B levels in adult patients with post-operative cognitive dysfunction (POCD).[25]

Azmitia *et al.* examined the effect of S100B overexpression on the behavior and morphology of neurons in mouse models. Their study showed that S100B excess caused cognitive deficits, obstacles to adapting, and decreased response to danger. In elderly mice, markers of apoptosis increased and signs of neuroinflammation occurred. In the end, transgenic S100B mice showed neurodegeneration and hyperphosphorylation of the structure of Tau, as seen in the late stages of Down syndrome and Alzheimer's disease.[11] However, in our study, there was no significant association between S100B levels and intelligence in children with Down syndrome.

In normal brain conditions, S100B is generally at the highest concentration at the beginning of development, decreases in adolescence and adulthood, and increases again with aging. Netto et *al.* found no correlation between S100B levels and age in children with Down syndrome, as found in normal children,[9] but in our study, there was a significant association between higher S100B levels and younger age. This different result may have been due to our differing age distribution.

This study had several limitations. The sample size was considered small for assessing for a possible association between variables. We also did not use a control group, so we could not compare to S100B levels in healthy individuals.

In conclusion, most children with Down syndrome have mild mental retardation and S100B levels above the normal range. However, there is no association between S100B levels and intelligence level in children with Down syndrome. There is an association between serum S100B levels in Down syndrome patients with age, as it is in normal individuals. Further studies with a larger sample size and control group should be conducted to confirm these findings.

Conflict of interest

None declared.

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Original Article

Macronutrient content in preterm and full term human milk in the first three weeks after delivery

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Abstract

Background The macronutrients in human milk change dynamically and vary among mothers. Evaluation of macronutrient content in human milk is needed to improve nutritional management in preterm infants.

Objective To measure the macronutrient content in preterm and full term human milk during three lactation periods in the first three weeks after delivery.

Methods We conducted a prospective study among 80 mothers of infants who were hospitalized in the Department of Perinatology/NICU at Sardjito Hospital, Yogyakarta. Carbohydrate, fat, protein, and caloric content were measured using a MIRIS human milk analyzer, once per week for three consecutive weeks after delivery. A single, daytime human milk specimen was collected in the morning by directly expressing from the breast.

Results Median protein, fat, carbohydrate, and caloric contents of mature milk in the preterm group were 1.40 (IQR 0.38), 3.25 (IQR 1.00), 5.70 (IQR 0.80) g/dL, and 60 kcal/dL, respectively. Median protein, fat, carbohydrate, and caloric contents of mature milk in the full term group were 1.40 (IQR 0.35), 3.30 (IQR 0.77), 5.80 (IQR 0.75) g/dL, and 62 kcal/dL, respectively, at the third week after delivery. In both groups, protein content in the first week was significantly higher than in the third week ($P<0.001$) after delivery. In contrast, fat content in the first week was significantly lower than in the third week ($P< 0.05$) after delivery, in both groups.

Conclusions There are no significant differences in macronutrient and caloric content between preterm and full term human milk during the first three weeks after delivery. However, there are significant changes in fat and protein content in both preterm and full term human milk during early lactation, between the first and third weeks. **[Paediatr Indones. 2019;59:130-8; doi: http://dx.doi.org/10.14238/pi59.3.2019.130-8]**.

Keywords: *human milk; macronutrient; calorie; lactation; preterm*

Human milk provides macronutrients, micronutrients, and bioactive substances needed by full term and preterm infants in early life.[1] Human milk macronutrients play an important role in the growth and development of infants and they are needed daily in larger quantities than other substances. Macronutrients in human milk consist of carbohydrates, fats, and proteins.[2] The *American Academy of Pediatrics* has recommended both preterm and full term infants to receive human milk from their own mothers, or from donors after it has been pasteurized according to standard procedures.[3] Human milk is a better choice than formula milk because it reduces the risk of necrotizing enterocolitis and sepsis.[1]

Protein and fat vary in concentration in preterm human milk, especially in the first four weeks after delivery.[4] Protein in preterm human milk decreases from 1.9 g/dL on the first day to 1.5 g/dL on days 22-30 after delivery, while the protein and caloric requirements for preterm infants increase by age.[5] Macronutrient content in preterm and full term

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human milk is relatively similar by 4-6 weeks after delivery.[6]

There have been few studies on macronutrient and caloric content of human milk at early lactation, or on changes between lactation stages in preterm and full term human milk in Indonesian mothers. The estimated human milk macronutrient content in our practice usually refers to published values from developed countries. Detailed information about macronutrient and caloric content in human milk may allow targeted nutritional management and individual fortification, especially for preterm and low birth weight infants.[7] A human milk analyzer (HMA) is designed to measure macronutrients (carbohydrates, proteins, and fats) and calories in 60 seconds, with a minimum 1-2 mL specimen, based on the principle of mid-infrared spectroscopy.[8] The HMA is essentially a portable and simple device for analyzing human milk and can be used to provide rapid measurement of milk composition.[9] This machine is especially advantageous for mothers of preterm infants, who often produce only small amounts of milk in early lactation.[10]

The primary objective of this study was to investigate the difference in macronutrient and caloric content of preterm and full term human milk, in the first three weeks after delivery. The secondary aim of this study was to study the difference in human milk macronutrient content between the lactation periods of colostrum (in the first week) and mature human milk (in the third week). The results are expected to provide preliminary data on macronutrient content of human milk from mothers in Yogyakarta, Indonesia.

Methods

Study subjects were lactating mothers who gave birth to preterm infants (gestational age 28 to < 37 weeks) or full term infants at the Department of Perinatology/ NICU, Dr. Sardjito Hospital, Yogyakarta. From July 2016 to September 2016, a total of 80 lactating mothers were recruited who met the inclusion criteria. General information on demographic factors, anthropometry (height and body weight before pregnancy, and at the time of data collection), and information on health-related problems (hypertension, diabetes, or heart disease) was collected by interviewing the participants. Further data on pregnancy outcomes such as gestational age at delivery (weeks), infant diagnosis, and neonatal birth weight (g) was obtained from medical records.

Study protocols and consent forms were approved by the Medical and Health Research Ethics Committee of Universitas Gadjah Mada Medical School/Dr. Sardjito Hospital. All participants provided written informed consent to participate in the study. There were 39 women in the preterm human milk group and 41 women in the full term human milk group.

We examined colostrum (i.e human milk, produced in low quantities in the first few days postpartum, is rich in immunologic components such as secretory IgA and lactoferrin), transitional milk (i.e human milk, typically produced from 7 days to two weeks), and mature milk (i.e. human milk, produced from the fourteenth day after delivery). Each participant provided breast milk once per week on day 3-7 the first week, day 8-14 the second week, and day 15-21 the third week after delivery. Breast milk was obtained by hand or pump. After each expression, if not immediately processed, milk specimens were poured into sealed containers and stored at 4°C. All specimens were processed and analyzed within 24 hours. Specimens which are not stored in ice-packed container were analyzed within 2 hours of being expressed.

Human milk specimens were from one expression, during the day, from one breast that had not been nursed for 2-3 hours. The mammary glands were fully evacuated of all accumulated milk in order to prevent any differences between foremilk and hindmilk. Specimens were homogenized and aliquoted. Surplus milk was returned to the infant.

To minimize diurnal variation in milk fat, sample collection was performed in the morning between 6:00AM and 8:00AM.[11] Mothers were encouraged to breastfeed or express breastmilk 8-12 times per day to enhance milk production. If the baby or mother had been discharged from the hospital, we visited their home at the time of collection and took 10 mL milk specimens within 1 hour. Specimens were labeled and sealed in a clean bottle, stored in an ice-packed container (about -15 to 4°C), then taken immediately to Dr. Sardjito Hospital for evaluation.

Three mL of milk was heated in a 40°C water bath, homogenized by gentle inversion of the container,

then subjected to measurement of fat, protein, carbohydrate, and energy content by a MIRIS mid-infrared human milk analyzer (HMA). Daily internal calibration was performed on the HMA using the check solution provided by the manufacturer (MIRIS). Since we only use fresh milk samples, the MIRIS HMA was operated using the unhomogenized sample mode, according to manufacturer's recommendations. The milk specimen (1 mL) was injected into the flow cell and measured for 60 seconds.

Human milk protein, fat, carbohydrate, and caloric contents are presented in mean and standard deviation or median and interquartile range in the tables, with 95% confidence intervals. Given the non-normal distribution of data, statistical analysis was performed by non-parametric test. Mann-Whitney test was used to analyze differences between non-related groups. Friedman and Wilcoxon tests were used to analyze differences between related groups. We used the *Statistical Package for the Social Sciences* (SPSS) program *version 22* software for statistical analysis. Results with P values < 0.05 were considered to be statistically significant.

Results

A total of 226 fresh human milk specimens were collected in the study, including 80 specimens collected in first week, and 73 samples collected each week in the second and third weeks after delivery. Three infants in the preterm group died less than 7 days after delivery and there was insufficient data for 4 infants in the full term group due to their being lost to follow-up. Subjects' flow chart is presented in **Figure 1**.

Table 1 shows the baseline characteristics of subjects. The distribution of age and parity was relatively similar between groups. Infants' birth weights ranged from 760 to 3,000 grams in the preterm group and from 1,980 to 4,098 grams in the full term group.

Kolmogorov-Smirnov and Shapiro-Wilk normality tests revealed a non-normal distribution of data (P=0.000), so we used a non-parametric test for statistical analysis (P<0.05; 95%CI). **Table 2** shows the comparison of median macronutrient and caloric content of the preterm and full term groups

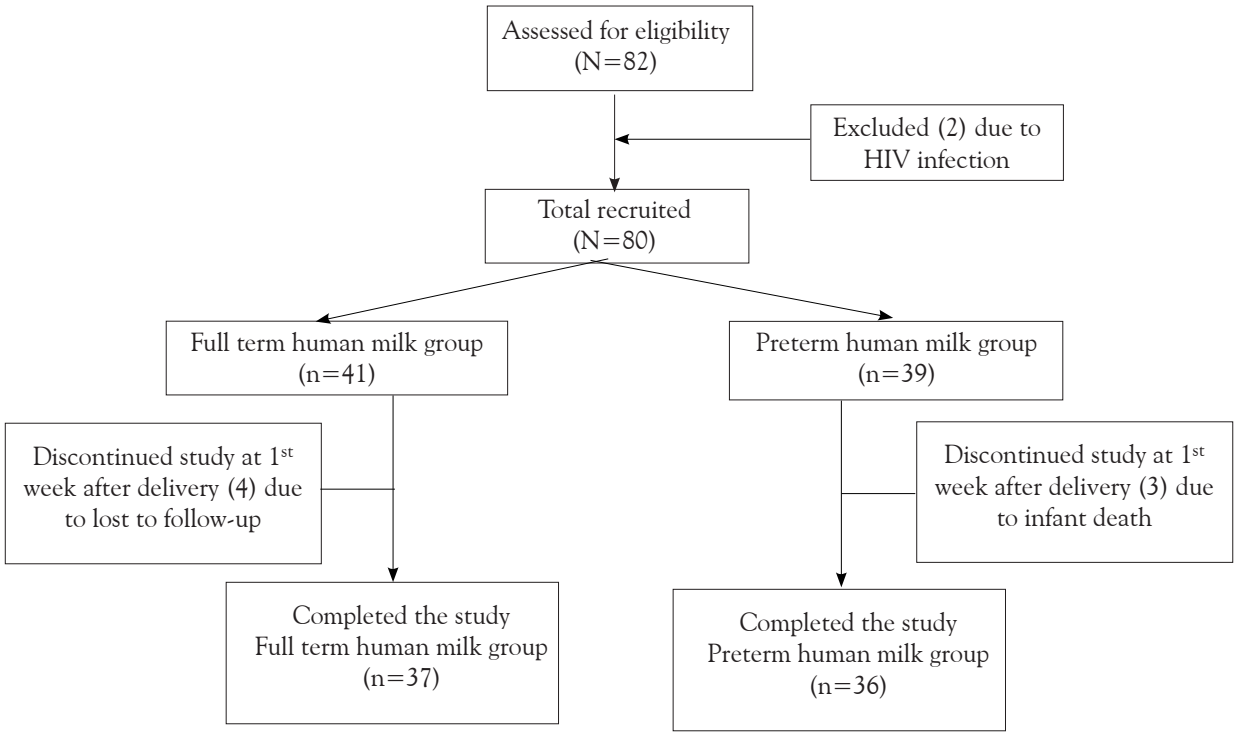

Figure 1. Study flow chart

at weeks 1, 2, and 3 after delivery. Based on non-parametric test, there was no significant difference in protein, fat, carbohydrate, or caloric content between the two groups, at any week during the study, respectively. In our study, there was high variability in the macronutrient content in both groups, on a week-to-week basis. In preterm human milk, the median protein contents (range) were significantly different on weeks 1, 2, and 3 [2.1 (1.0-6.1) g/dL, 1.5 (0.7-4.1) g/dL, and 1.4 (0.4-3.9) g/dL, respectively, (P=0.003)]. Median fat contents (range) in preterm human milk on weeks 1, 2, and 3 were also significantly different [2.3 (0.7-4.0) g/dL, 2.7 (1.1-5.3) g/dL, and 3.25 (1.4-5.6) g/dL, respectively, (P<0.001)]. However, median

Table 1. Subjects' baseline characteristics

Characteristic	Preterm group (n=39)	Full term group (n=41)
Median maternal age (range), years	30 (17-41)	31 (20-41)
Median parity (range)	2 (1-4)	2 (1-4)
Median gestational age (range), weeks	34 (28-36)	39 (37-41)`
Median maternal weight increase during pregnancy (range), kg	9 (0.5-23)	10 (3-28)
Infant birth weight, n (%)		
<1,000 grams	2 (5)	0
1,000-1,499 grams	13 (33)	0
1,500-2,499 grams	21 (54)	3 (7)
2,500-4,000 grams	3 (8)	37 (90)
>4,000 grams	0	1 (2)
Median body mass index (range), kg/m2	22.31 (16.65-32.47)	24.22 (16.64-34.22)
Delivery method, n (%)		
Ceasarian section	18 (46)	20 (49)
Vaginal	21 (54)	21 (51)
Birth location, n (%)		
Sardjito Hospital	36 (92)	36 (88)
Outside Sardjito Hospital	3 (8)	5 (12)

Table 2. Comparison of human milk macronutrient and caloric content

Parameters	Preterm group	Full term group	P* value
Median protein (IQR), g/dL			
Week 1	2.10 (1.0-6.1)	1.80 (1.3-4.2)	0.63
Week 2	1.50 (0.7-4.1)	1.60 (1.1-4.9)	0.41
Week 3	1.40 (0.4-3.9)	1.40 (0.8-4.2)	0.32
P**	0.003	<0.001	
Median fat (IQR), g/dL*			
Week 1	2.30 (0.7-4.0)	2.60 (0.9-6.7)	0.12
Week 2	2.70 (1.1-5.3)	2.75 ((1.3-5.9)	0.87
Week 3	3.25 (1.4-5.6)	3.30 (1.3-5.6)	0.84
P**	<0.001	0.045	
Median carbohydrate, g/dL			
Week 1	5.60 (3.1-8.8)	5.70 (4.8-7.2)	0.28
Week 2	5.70 (4.3-6.4)	5.70 (4.2-6.9)	0.75
Week 3	5.70 (4.3-7.9)	5.80 (3.9-7.1)	0.77
P**	0.365	0.382	
Median calories (IQR), kcal/dL			
Week 1	56.00 (41-84)	58.00 (43-102)	0.07
Week 2	58.50 (43-80)	58.00 (46-87)	0.95
Week 3	60.00 (46-100)	62.00 (43-87)	0.52
P**	0.029	0.032	

*Mann Whitney test, ** Friedman test, IQR = interquartile range

carbohydrate contents (range) in preterm human milk on weeks 1, 2, and 3 were not significantly different [5.6 (3.1-8.8) g/dL, 5.7 (4.3-6.4) g/dL, and 5.7 (4.3-7.9) g/dL, respectively, (P=0.365)].

In full term human milk, median protein contents (range) significantly differed on weeks 1, 2, and 3 [1.8 (1.3-4.2) g/dL, 1.6 (1.1-4.9) g/dL, and 1.4 (0.8-4.2) g/dL, respectively, (P<0.001)]. Median fat contents (range) in preterm human milk also significantly differed on weeks 1, 2, and 3 [2.6 (0.9-6.7) g/dL, 2.75 (1.3-5.9) g/dL, and 3.3 (1.3-5.6) g/dL, respectively, (P=0.045)]. However, median carbohydrate contents (range) in full term human milk were not significantly different in weeks 1, 2, and 3 [5.7 (4.8-7.2) g/dL, 5.7 (4.2-6.9) g/dL, and 5.8 (3.9-7.1) g/dL, respectively, (P=0.0382)].

Table 2 shows that the macronutrient composition changed with increasing post-natal age. In both the full term and preterm groups, a significant decline in protein content occurred over the first 3 weeks. In contrast, fat and caloric content increased with post-natal age (P<0.05).

Table 3 presents the comparison of macronutrient and caloric contents between colostrum (week 1) and mature milk (week 3) in the preterm (n=36) and full term (n=37) groups. Human milk composition changes during the days of early lactation, as an effect of milk maturation. There were significant differences in protein and fat content between the first and third weeks after delivery, in both groups.

In **Tables 2** and **3**, the caloric content in the preterm and full term groups were 56-60 kcal/dL and 58-62 kcal/dL, respectively, for every 100 mL of human milk. Both were lower than published reference values.[12]

Discussion

The macronutrient composition of human milk varies in response to several factors, including maternal age, maternal condition, and infant condition.[1] Human milk macronutrient content may vary between lactation times both within an individual and among individuals. Macronutrient content has been associated with maternal characteristics, lactation/feeding frequency, lactation stage, storage period, and pasteurization process.[13,14] Lactation can be divided into three stages, based on the length of time after delivery as follows: 1) colostrum, produced in low quantities in the first few days postpartum, is rich in immunologic components such as secretory IgA and lactoferrin, 2) transitional human milk, typically produced from 7 days to two weeks, and 3) mature human milk, produced from the fourteenth day after delivery.[1] Human milk is considered to be fully mature in the fourth to sixth weeks after delivery, and in this period the macronutrient and caloric content is relatively similar over lactation times and among individuals.[2]

Table 3. Comparison of macronutrient and calorie content on weeks 1 and 3 after delivery

Parameters	Week 1 (colostrum)	Week 3 (mature human milk)	P*** value
Median protein (IQR), g/dL			
Preterm	2.10 (1.0-6.1)	1.40 (0.4-3.9)	<0.001
Full term	1.80 (1.3-4.2)	1.40 (0.8-4.2)	<0.001
Median fat (IQR), g/dL*			
Preterm	2.30 (0.7-4.0)	3.25 (1.4-5.6)	<0.001
Full term	2.60 (0.9-6.7)	3.30 (1.3-5.6)	0.03
Median carbohydrate, g/dL			
Preterm	5.60 (3.1-8.8)	5.7 (4.8-7.2)	0.12
Full term	5.70 (4.3-7.9)	5.80 (3.9-7.1)	0.64
Median calories (IQR), kcal/dL			
Preterm	56.00 (41-84)	60.00 (46-100)	0.01
Full term	58.00 (43-102)	62.00 (43-87)	0.01

*Mann Whitney test, ** Friedman test, IQR = interquartile range

In most previous studies, preterm breast milk had higher content of various nutrients, especially protein, compared to corresponding values in full term human milk.[15] Gidrewicz *et al.* performed a meta-analysis of 41 studies on human milk macronutrients and micronutrients, in North America, Europe, and Japan. They reported a significant difference in protein content between preterm and full term human milk on days 0-3 and the second week after delivery. The protein content in preterm human milk on days 0-3 and the second week is 2.7 g/dL and 1.1 g/dL, respectively. Whereas in full term human milk, the protein content on days 0-3 and the second week is 2.0 g/dL and 1.0 g/dL, respectively.[6] Bauer *et al.* undertook a longitudinal study in Germany for 8 consecutive weeks postpartum and reported that carbohydrate, lactose, calorie, and fat contents were significantly higher in preterm human milk than in full term human milk.[16] However, we did not find significant differences in protein, fat, carbohydrate, or caloric content between preterm human milk and full term milk in the first three weeks after delivery.

Paul et al. used a longitudinal cohort design to compare human milk content on days 1, 7, 14, and 21 after delivery and reported no significant difference in macronutrients between the full term and preterm groups. The study involved 52 subjects, consisting of 23 mothers of full term infants and 29 mothers of preterm infants in India. They also reported a significant decrease in protein content and a significant increase in fat content in the third week after delivery.[17] Our study was of similar design, in that our sampling method was a single expression of milk, not pooling of milk in a 24-hour period. Hsu *et al.* in Taiwan reported that 17 mothers with infants of less than 35 weeks' gestational age and 15 mothers with infants of full term gestational age had no significant differences in protein, fat, lactose, caloric, calcium, or phosphate content of human milk in the first week and fourth week after delivery.[18]

In our study, high variability in the HMA results in both groups lead to non-normal data distribution. Kreissl *et al.* in Austria reported that HMA results on 83 human milk specimens from mothers of preterm infants on the second to the fourth weeks after delivery also had a non-normal data distribution, with median protein, fat, carbohydrate, and caloric content of 1.0 (0.2-2.2) g/dL, 3.1 (1.1-6.1) g/dL, 6.6 (5.5-8.0) g/dL, and 59 (39-94) kcal/dL, respectively, for every 100 mL of human milk.[19] The similarity between our study and theirs was the homogenization technique, which was performed manually, while the differences were subject demography (enrolled from a developed vs. developing country) and the 24-hour pooling sampling method in their study.

Our measurement of carbohydrate content by the MIRIS HMA resulted in data with fairly high variation, similar to previous research by Gidrewicz *et al.*,[6] Silvestre *et al.*,[20] and Fusch *et al.*[21] Fat content was the most varied component of human milk macronutrients in 24 hours compared to protein and carbohydrate contents in an individual.[21,22] Fat is the major component of calories in human milk, since 1 gram of fat contains an equivalent 10 kilocalories. As such, calculation of calories in milk depends on factors that affect fat examination. Khan et al. reported the effect of a 24-hour pooling sample method on the results of caloric and fat content. Fat content was higher in specimens collected from mothers who frequently breastfed and by fully evacuating the breast of milk.[21]

Protein and carbohydrate contents in milk were not related to the volume of milk produced, the nursing process, diurnal variation, or maternal nutritional status.[22] When collecting human milk only a single time in 24 hours, the sampling time and lactation interval should be standardized. The best time to collect human milk is 6:00 to 8:00AM when the fat content is most in accordance to a 24-hour pooled milk specimen.11 This sampling method, however, explains only 55% of the total variance in the 24-hour fat content of breast milk. A standard sampling time and lactation interval could reduce fat content variation among individuals.[22]

The recommended homogenization technique prior to macronutrient measurement by HMA is using an ultrasonic vibrator for at least 30 seconds per 1 mL sample, to minimize the presence of an unexamined fat matrix.[21] However, in our study, the fresh milk specimens had never been frozen or thawed, so according to the HMA MIRIS instructions, homogenization should be done manually by gentle inversion for 30-60 seconds.[23]

In addition, the variability in the milk composition may have been due to different laboratory methods. As such, the fat and carbohydrate contents in our

study were lower than the references. The HMA cannot distinguish between nutrient and non-nutrient content, as it only measures total protein, total fat, total carbohydrate, and total calories.[19] However, Groh-wargo *et al.* showed that results from a mid-infrared spectroscopy analyzer did not significantly differ compared to conventional methods for macronutrient measurement, i.e., Kjehdahl for protein, Mojonnier for fat, and liquid chromatography for lactose.[8]

We found a significant decrease in protein content and significant elevation in fat and caloric content in mature human milk compared to colostrum milk. Protein and fat content were strongly associated with lactation stage.6 High protein content in colostrum was consistent with the physiological stage of the first week, in which secreted milk contains a lot of protein in the form of immunoglobulin.[2] Increased fat content in more mature milk might be associated with increased intensity, frequency, and duration of nursing, in accordance with infant needs.[15] At the beginning of each lactation episode, foremilk consistency is usually more fluid and becomes more viscous at the end of lactation when hindmilk is produced.[5] There was a gradual increase in fat content in human milk in each episode of lactation, but there was no significant increase in protein and lactose contents. Fat content increases in the first 15-30 minutes of lactation, after which the fat content is relatively constant.[24]

Although the mechanism of differences in human milk protein content in preterm and full term infants is unclear, this difference was only significant in the first month after delivery. Variations in protein, fat, and mineral contents in human milk in the first four weeks after delivery were higher in the preterm than in the full term milk groups.[5] A meta-analysis after the fifth week after delivery found no difference in protein content between preterm and full term human milk groups.[6]

In our study, we calculated the mean of human milk protein content in the preterm on week 1, week 2, week 3, were 2.2 g/dL, 1.6 g/dL, 1.5 g/dL, respectively. These values were within the normal range, as per the *American Academy of Pediatrics* reference (week 1: 0.3-4.1 g/dL, week 2: 0.8-2.3 g/dL, week 3: 0.6-2.2 g/dL). In the full term, the mean of human milk protein content on week 1, week 2, week 3 were 1.9 g/dL, 1.6 g/dL, 1.5 g/dL, respectively. According to *American Academy of Pediatrics* reference (week 1: 0.4-3.2 g/dL, week 2: 0.8-1.8 g/dL, week 3: 0.8-1.6 g/dL), these values were also within the normal range. However, mean caloric content in mature human milk was lower than the references (preterm: 62.8 kcal/dL and full term: 62.7 kcal/dL, per 100 mL). In a meta-analysis conducted in developed countries, the mean caloric content of pretem and full term human milk were 66 kcal/dL and 77 kcal/dL, respectively.[6] In clinical practice, a reference standard of 65-70 kcal/dL per 100 mL of human milk is used for planning and evaluating infant nutrition,[12] which was a higher value than our findings.

We did not evaluate for an association between in macronutrient content and infant growth and development, nor the human milk volume produced day per day by mothers. In clinical practice, infant weight gain, especially in preterm infants, is an important parameter for evaluating sufficiency of human milk intake, as well as the response to changes in macronutrient content from colostrum to mature human milk.[15] Further studies are needed to investigate for possible associations. Another limitation was our sample size, however, it was estimated to have 80% power to detect a difference of mean between groups greater than one standard deviation. Differences in macronutrient content between preterm and full term milk groups might be detected with a larger sample size. The other limitations of our study were the sampling method and the duration of investigation. We did not perform 24-hour human milk pooling collection, as in other studies, although we attempted to standardize breast expression method, sample collection time, and lactation interval. Also, the observation period was not long enough, as full maturation of human milk is achieved at weeks 4-6 after delivery, so we may not have fully described the changes in macronutrient content over lactation stages in our Indonesian population.

Our results may be used as preeliminary data on macronutrient and caloric content of human milk in the first three weeks after delivery in the maternal population in Yogyakarta. It can also be the basis for further study with a greater number of subjects, longer data collection period, better sampling techniques and collection, and more complete analysis of maternal and infant characteristics and nutrition factors.

In conclusion, there is no significant difference

in protein, carbohydrate, fat, and caloric contents of preterm and full term human milk in the first three weeks after delivery. Mature milk has higher fat content, but lower protein content than colostrum milk. In our population, a lower caloric content in human milk than the published value is noted in both preterm and full term human milk. Therefore, monitoring of an infant's body weight gain might be indicated, especially for high risk populations, i.e., preterm and very low birth weight infants. If fortification of human milk is needed, it should be individualized according to the macronutrient variability in milk over lactation periods.

Conflict of interest

None declared.

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Original Article

Highly active antiretroviral therapy and left ventricular diastolic function in children with human immunodeficiency virus infection

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Abstract

Background In the past, cardiovascular involvement did not seem to be a common complication of HIV, but in recent years it has been described more frequently. With the advent of highly active antiretroviral therapy (HAART), the symptoms of cardiac disease has changed, as the number of HIV-infected patients with abnormal diastolic parameters has increased significantly, often presenting as symptomatic rather than asymptomatic.

Objective To analyze for a possible correlation between HAART duration and left ventricular diastolic function in HIV-infected children.

Methods This cross-sectional study was conducted from December 2016 to December 2017 at the Cardiology and Allergy-Immunology Division/Department of Child Health, Universitas Udayana Medical School/Sanglah Hospital, Denpasar, Bali. Subjects with HAART were collected using a consecutive sampling method. The following data were recorded for each subject: age, sex, current stage of HIV, CD4+ level, as well as HAART regimen and duration of use. Transthoracic echocardiography was performed for tissue doppler imaging (TDI) of diastolic function. Spearman's test was used to analyze the strength of correlation based on normality test results.

Results This study involved 53 subjects, 21 of whom had impaired diastolic function. There was no correlation between HAART duration and diastolic function in children with HIV infection (r= -0.03; P=0.82).

Conclusion Diastolic dysfunction is found in children under HAART treatment, but there is no correlation between HAART treatment duration and diastolic dysfunction. **[Paediatr Indones. 2019;59:139-43; doi: http://dx.doi.org/10.14238/pi59.3.2019.139-43]**.

Keywords: *HIV; left ventricular diastolic function; HAART*

Human immunodeficiency virus (HIV) is an important cause of childhood morbidity and mortality, affecting more than 1.3 million children worldwide.[1] With the advent of highly active antiretroviral therapy (HAART), HIV infection has become a chronic disease with longer life expectancy.[2] Cardiac diseases are frequent complications in these patients.[2] The potential mechanisms of cardiac complications in HIV include, but are not limited to, direct cardiotoxicity by the virus itself, immune-mediation mainly by cytokines, nutritional deficiencies, and antiretroviral medications.[3]

Some of the medicines used to treat HIV infection may have a deleterious effect on the myocardium. Mitochondrial toxicity is an acknowledged side effect of HAART.2 Defects in mitochondrial DNA (mtDNA) replication and decreased energetics are caused by zidovudine, as well as other nucleoside reverse transcriptase inhibitors (NRTI). The spectrum of cardiac disease varies significantly between the

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pre-HAART and post-HAART eras.[4] In the pre-HAART era, HIV-associated cardiomyopathy was defined as symptomatic, systolic dysfunction with dilated left ventricle, and seen almost exclusively in patients with advanced clinical stage of HIV and low CD4 levels.[2,5] In the post-HAART era, the symptoms of the cardiac disease has changed into diastolic dysfunction, and the condition has changed from mostly symptomatic to asymptomatic. Chelo *et al.* showed that from 100 children with HIV, 32% had LV diastolic dysfunction.[6]

Prior studies have reported that HAART may contribute to impaired diastolic function in adults. However, this effect is unclear in children. A previous study reported only reduced diastolic function in HIV-negative children exposed to HAART in utero.[7] Hence, we aimed to assess for a correlation between left ventricular diastolic function in HIV-infected children and HAART duration of use.

Methods

This cross-sectional study was done from December 2016 to December 2017, at the Cardiology and Allergy-Immunology Division/Department of Child Health, Universitas Udayana Medical School/Sanglah Hospital Denpasar, Bali. Target populations were inpatient and outpatient children with HIV infection in Sanglah Hospital. Informed consent was obtained from the parents. The inclusion criteria were patients on HAART and aged under 18 years. Patients on medications with known cardiovascular effects (such as antiarrhythmic drugs, theophylline, and adriamycin), patients with pre-existing cardiac diseases, or poor adherence to HAART were excluded.

Subjects were consecutively enrolled until the required sample size was complete. Sample size was calculated using the formula based on consideration of analytic, single group, unpaired, two-tailed study, with alpha 0.05, power 80, and correlation score 0.4 based on clinical judgment. The minimum required sample size was calculated to be 48. The total number of subjects obtained was 53.

Subjects underwent uniform clinical evaluations and the following data were recorded: age, sex, current stage of HIV, CD4+ level, as well as HAART regimen and duration. Transthoracic echocardiography was performed and interpreted by a cardiologist. All studies were performed on a *General Electric Vivid 7* ultrasonograph with 3s-MHZ or 7s-MHZ transducers. Tissue doppler imaging (TDI) was obtained using pulsed-wave tissue Doppler. Pulsed wave TDI velocity measurements were obtained by placing the sample volume at the mitral annular level from the septal annulus. The TDI signal over a cardiac cycle has three peaks: a positive systolic peak and two negative diastolic peaks. The negative waves represent the early diastolic myocardial relaxation (é velocity) and active atrial contraction in late diastole (á velocity). The TDI é septal velocity was recorded. The variables were defined as follows:

- Left ventricular diastolic function was represented by TDI é medial, as measured from the septal annulus.[8] A medial é velocity < 12 cm/s indicated impaired LV diastolic function or cardiomyopathy.[9]
- HIV clinical stages were categorized as 1 through 4 based on *World Health Organizatio*n (WHO) 2014 guidelines.[10]
- Highly active antiretroviral therapy (HAART) was defined as any regimen that included three antiretroviral drugs from 2 or more antiretroviral drug classes [nucleoside reverse transcriptase inhibitors (NRTI), nonnucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitors (PI)]. The first line therapy typically consists of 2 NRTI and 1 NNRTI.[10] The duration of HAART was the time from the start of HAART use to the time of the study (months).
- Adherence to HAART was defined as a patient's ability to follow a treatment plan, as well as take medications at prescribed times and frequencies.[11] Data were obtained by parents' or caregivers' reports.
- The CD4 data were obtained from within three months of subjects' echocardiography examination.

Characteristics of subjects are presented descriptively in table and narrative form. The data distribution was analyzed with Kolmogorov-Smirnov test because the sample size was more than 50, and considered to be normally distributed if P value > 0.05. Normality test of HAART duration and TDI é medial results revealed an abnormal data distribution. After data transformation by log10, HAART

duration data were still abnormally distributed and nonparametric, with P value < 0.05. Data were numeric and Spearman's test was used to analyze the strength of correlation based on normality test results. Statistical analysis was performed with *SPSS version 18* software. This study was approved by the Medical Ethics Committee of Universitas Udayana, Sanglah Hospital, Denpasar.

Results

Between December 2016 and December 2017, 66 children with HIV participate in the primary study. Twelve children did not consume HAART and 54 subjects fulfilled the inclusion criteria for this study. One subject was excluded because of improper adherence due to an allergic reaction. Fifty-three subjects underwent echocardiography and retained for analysis.

Table 1. Subjects' characteristics

Clinical characteristics	¯(N = 53)
Age, n (%)	
≤ 5 years	12 (22.6)
> 5 years	41 (77.3)
Male, n (%)	33 (62.2)
Current stage of HIV, n (%)	
Stage I	43 (81.1)
Stage II	4 (7.5)
Stage III	4 (7.5)
Stage IV	2 (3.7)
CD4+ level (age ≤ 5 years), n	12
≥15%	8
<15%	4
CD4+ level (age > 5 years), n	41
≥ 200 cell/mm^3	28
< 200 cell/mm^3	13
HAART regimen, n (%)	
First line	45 (84.9)
Second line	8 (15.0)
Median duration of HAART use (range), months	48 (2-120)
Median TDI é medial (range), cm/s	12 (8-16.6)

The characteristics of subjects are described in **Table 1**. The age range of the subject was 2 to 14 years, with median age of seven years. A total of 81.1% subjects were HIV stage one. The frequency of subjects using a second line HAART regimen was 15%. More than 50% of subjects had absolute CD4+ levels above 200 cell/mm^3 and percentages above 15%. There were 21 subjects with TDI é medial less than 12 cm/s.

Spearman's test was used to analyze for a possible correlation between duration of HAART and TDI é medial. The two variables had a negative association and very weak correlation, with r=-0.03, but were not statistically significant (P=0.82) (**Table 2**).

Discussion

The subjects in our study ranged from 2 to 14 years in age. Most were above five years and had stage 1 infection. More than 50% of subjects had CD4+ level ≥15% or ≥ 200 cells/mm^3. The overall data indicated that subjects had good control of the disease.

The longest duration of HAART usage was 120 months, and the shortest was two months. Longer HAART duration indirectly showed an increase in life expectancy among children with HIV. This result was in agreement with that of Ewings et al. who noted that the proportion of patients expected to survive 5, 10, and 15 years after seroconversion in the HAART era were 99%, 93%, and 89%, respectively.[12]

The risk of premature cardiovascular disease has been associated with specific antiretroviral therapies.[13] Nucleoside reverse transcriptase inhibitors (NRTIs) have been associated with mitochondrial toxicity.[7] Zidovudine may inhibit cardiac mitochondrial DNA polymerase and induce ultrastructural changes in cardiac myocytes.[14] Protease inhibitors (PIs) have also been implicated in adversely affecting cardiac function and atherogenic risk in both adults and children. The PI-containing regimens have specifically been

Table 2. Correlation between HAART duration and diastolic function

Variables	Median (range)	Correlation coefficient	P value
Duration of HAART use, months	48 (2-120)	-0.03	0.82
TDI é medial, cm/s	12 (8-16.6)		

associated with an increase in LV mass and diastolic dysfunction in adults.[7]

In our study, HAART regimens used in our setting were categorized as first line or second line. The first line regimen included two NRTIs (zidovudine and lamivudine) and one NNRTI (nevirapine), and was most commonly used by our subjects. Eight subjects with previous treatment failure had second line HAART regimen, which consisted of two NRTIs (tenofovir or abacavir and lamivudine) and one PI (lopinavir/ritonavir).

In the HAART era, the prevalence of systolic dysfunction has decreased and the number of patients with severely impaired ejection fraction is quite low. However, the number of HIV-infected patients with abnormal diastolic parameters has increased significantly.[2,5] Previous study evaluating the relationship between HAART and diastolic dysfunction in children with HIV has been limited. In 1992, Lipshultz *et al.* only evaluated the effect of zidovudine on systolic function and left ventricular dimension. Zidovudine was administered every 6 hours in that study, with conventional echocardiography used to evaluate the left ventricular dimension.[15] Kuswiyanto et al. (2011) also assessed left ventricular function disorder in children with HIV and its associations with CD4+ level and clinical stage, but not its association with HAART.[16]

Our study differs with others because we assessed for an association between HAART administration duration and diastolic function. Left ventricular diastolic function was assessed by TDI, which is more sensitive than conventional echocardiography for detecting early myocardial alterations. The TDI is useful for screening and detection of subclinical myocardial dysfunction.[8] We found 21 (39%) patients with diastolic dysfunction. Similiarly, Mondy et al. showed that 26% of HIV-infected subjects suffered from diastolic dysfunction.[17] However, the risk of left diastolic dysfunction in children with HIV was not significantly increased with longer HAART duration. An in vitro study by Lewis *et al.* demonstrated that zidovudine and HIV infection led to the independent development of cardiomyopathic changes in a transgenic mouse model. The dose of zidovudine used in that study was much higher than that used clinically in HIV-infected patients (~200 mg/kg *vs.* 8 mg/kg, respectively).[18] Studies in adults also revealed different results. Luo *et al.* showed that zidovudine exposure was associated with higher prevalence of diastolic dysfunction. A potentially important finding was that using zidovudine for more than 12 months showed a trend towards increased diastolic dysfunction.[19] Our study population, which were children, might explain the disparate results, as they are less likely to have risk factors for cardiovascular disorders than adults. Cardiac abnormalities in adults might result from the complex interactions among HIV infection itself, HAART medication, and other non-HIV related factors such as smoking, obesity, hypertension, and diabetes. Meng *et al.* also showed that HAART exposure was correlated with increased diastolic dysfunction in adults.[20] The different results may be related to the few patients who used PIs in our study (only eight subjects), compared to previous study.[7]

A limitation of this study was that adherence to the drug regimen was evaluated by parents' or family reports, without any validated methodology. Adherence affects the drug level in blood, thus potentially affecting study results. The diastolic dysfunction (cardiomyopathy) that occurred in HAART-treated children in this study was not correlated to HAART treatment duration. Our results suggest that HAART can be safely used in this population, even though regular monitoring of diastolic function should be considered. Further study is required to elucidate relationships between stratified variables and duration of HAART treatment, as well as comparison to a control group with no HAART history.

Conflict of interest

None declared.

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Original Article

Relationship between serum ferritin and zinc levels in pediatric thalassemia major patients

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Abstract

Background In thalassemia patients, reduced zinc absorption results from increased serum iron due to repeated blood transfusions, increased iron absorption due to ineffective erythropoiesis, and competitive inhibition between iron and zinc in binding to transferrin, a means of transporting both types of minerals in the blood. Few studies have been done to examine zinc levels in thalassemia patients and its relationship with ferritin.

Objective To compare serum zinc in thalassemia patients and healthy controls and to assess for a possible correlation between serum ferritin and zinc in thalassemia patients.

Methods This cross-sectional study in 68 subjects was done from October 2016 to August 2017. Serum ferritin measured by chemiluminescence immunoassay and serum zinc by inductively coupled plasma mass spectrometry (ICP-MS). Wilcoxon test was used to analyze for differences between serum zinc in thalassemia patients and controls. Spearman's correlation test was used to analyze for a possible correlation between ferritin and serum zinc in thalassemia patients.

Results There were 34 patients with thalassemia and 34 healthy control subjects. The median serum zinc was 119.34 μg/dL (IQR=71.27) in the thalassemia group and 120.08 μg/dL (IQR=26.28) in the control group (P=0.36). There was no significant correlation between serum ferritin and zinc in thalassemica children (r=-0.023; P=0.895).

Conclusion There is no difference in serum zinc levels between thalassemic children and healthy controls. There is no correlation between serum ferritin and zinc in thalassemica children. **[Paediatr Indones. 2019;59:144-9; doi: http://dx.doi.org/10.14238/pi59.3.2019.144-9]**.

Keywords: *HIV; thalassemia; ferritin; zinc*

Thalassemia is an inherited, autosomal recessive blood disorder characterized by abnormal hemoglobin synthesis that causes red blood cells to be easily damaged and fragile.[1] The main treatment for thalassemia is blood transfusion, in order to maintain hemoglobin levels above 10 g/dL.[2] Complications in thalassemia generally occur due to the disease itself (chronic anemia) and the main therapy, blood transfusion. Repeated blood transfusions cause iron accumulation in the tissues which can be aggravated by increased iron absorption due to ineffective erythropoiesis.[3,4]

Serum ferritin is used to monitor iron levels in the body. High levels of iron in children cause oxidative trauma and tissue siderosis. Complications, such as diabetes, liver cirrhosis, heart failure, hypothyroidism, short stature, and hypogonadism, often occur. In addition, osteoporosis, thromboembolism, zinc deficiency, and other complications can occur in patients with thalassemia.[5-7]

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Zinc is an important micronutrient found in almost every cell in the body. Zinc regulates enzyme systems that affect cell division and proliferation, wound healing, DNA synthesis, and maintaining normal structures and/or functions of several enzymes. Zinc is also very important for growth, puberty, and the immune system.[8,9] Several studies have been conducted on zinc deficiency in thalassemia major patients.[10-14] Further studies are needed to determine factors associated with low serum zinc levels and their relationship to serum ferritin levels of in such patients, hence, we aimed to evaluate serum zinc levels and its relationship to serum ferritin in children with thalassemia major treated at Dr. M. Djamil Hospital, Padang, West Sumatera.

Methods

This cross-sectional study was conducted from October 2016 to August 2017. Subjects were children in Dr. M. Djamil Hospital, Padang, West Sumatera, who fulfilled the inclusion criteria, and consisted of 34 thalassemia patients and 34 healthy children as controls. The inclusion criteria were thalassemia patients who were routinely transfused and whose parents agreed to take part in the study. The control group consisted of healthy children that had been adjusted for age and sex with the thalassemia patients.

Serum ferritin measured by chemiluminescence immunoassay and serum zinc by inductively coupled plasma mass spectrometry (ICP-MS). Data analysis and processing was done using *SPSS version 21* software. Wilcoxon test was used to analyze for differences between serum zinc in thalassemia patients and controls. Spearman's correlation test was used to analyze for a possible correlation between ferritin and serum zinc in thalassemia patients.

Study protocols and consent forms were approved by the Medical and Health Research Ethics Committee of Universitas Andalas Medical School/ Dr. M. Djamil Hospital.

Results

The 68 subjects, 34 thalassemic and 34 healthy children, categorized by age, namely, ≤10 years and >10 years. Most subjects were aged ≤ 10 years (20 children in each group, 58.8%). There were 10 children (29.4%) with undernourished nutritional status in the thalassemia group and 8 (23.5%) in the control group. No subjects had malnutrition. The highest incidence of stunting was 20 children (58.8%) in the thalassemia group, while the control group had 6 children (17.6%) with stunting. The characteristics of study subjects are shown in **Table 1**.

As shown in **Table 2,** the median zinc level in the thalassemia group was 119.34 μg/dL, whereas that in the control group was 120.08 μg/dL. Wilcoxon signed rank test revealed no significant difference in zinc levels between groups (P=0.36).

Statistical analysis of ferritin and serum zinc levels in thalassemia patients was tested done with Spearman's correlation test. Ferritin and serum zinc was not significantly correlated in thalassemia patients, as shown in **Figure 1**.

Table 1. Characteristics of study subjects

	Thalassemia group (n=34)	Control group (n=34)
Sex, n		
Male	18	20
Female	16	14
Age, n		
≤ 10 years	24	20
> 10 years	10	14
Nutritional status, n		
Well-nourished	24	21
Undernourished	10	8
Overweight	0	1
Obese	0	4
Stunting, n	20	6

Table 2. Serum zinc levels in the thalassemia and control groups

Groups	Zinc level			
	n	Median	IQR (range)	P# value
Thalassemia	34	119.34	71.27 (87.42-330.85)	0.36
Control	34	120.08	34 (26.28-91.26)	

Serum zinc units=μg/dL; #Wilcoxon signed ranks test

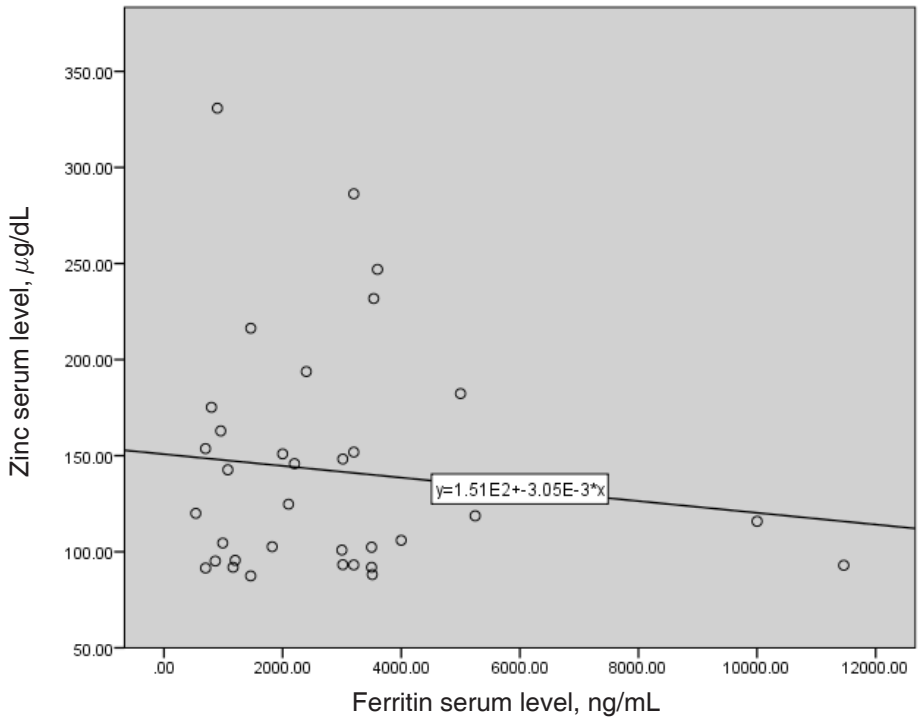

Figure 1. Correlation between ferritin and serum

Discussion

The two subject groups were categorized by age, either ≤ 10 years or > 10 years. The growth pattern in children with thalassemia who receive routine blood transfusions to maintain hemoglobin levels of more than 9 g/dL is relatively normal until the age of 9 to 10 years. Subsequently, regular transfusions cause iron overload, which triggers tissue damage, due to accumulation of free radicals in the organs. Thus, the speed of growth begins to decline, causing short stature and growth failure.[15,16]

There were 26 children with stunting, 20 in the thalassemia group and 6 in the control group. Growth disorders are quite common complications in children with thalassemia major. Tissue hypoxia due to chronic anemia, micronutrient deficiency, inadequate blood transfusion, iron buildup in endocrine organs, and hepatosplenomegaly cause a decrease in appetite so that food intake is reduced, resulting in nutritional disorders and failure to thrive in thalassemia patients.[17] Side effects from iron chelation therapy are also causes of growth disorders in patients with major thalassemia.15 The percentage of patients with stunting in our study was associated with the incidence of iron overload, in which 95% of stunting occurred in patients with ferritin levels >1,000 ng/mL.

Several studies have linked growth disorders in thalassemia patients with zinc deficiency. Arcasoy et al. showed that zinc deficiency is one of the factors responsible for delayed growth in thalassemia patients.[18] However, Mehdizadeh *et al.*,[19] Arijanti *et al.*,[20] and Eshghi *et al.*,[21] found no significant relationship between zinc levels and short stature. Faranoush et al. compared thalassemia patients who received zinc supplementation at a dose of 60 mg/day for 18 months, to thalassemia patients who did not get zinc. They showed that zinc supplementation was only useful in patients with zinc deficiency, otherwise zinc prophylaxis had no effect on growth.[22]

In several previous studies, serum zinc deficiency in thalassemia patients was associated with the incidence of chronic hemolysis, hyperzincuria, low intake of zinc in food, high iron content, and iron chelation. Arijanti et al. found that all subjects had zinc deficiency.[20] Arcasoy et al. noted a decrease

in zinc levels in plasma, erythrocytes, and hair, and increased zinc excretion in urine of thalassemia patients.[18] Hess *et al.* in Iran found that in 40 pediatric thalassemia subjects, more than 65% of patients with hypozincemia had zinc concentrations below 70 μg/dL.[23] Nidumuru *et al.* compared serum zinc levels in 35 thalassemia patients and to 35 healthy controls and reported that 65% of thalassemia patients had zinc deficiency.[12]

In our study, no subjects had zinc deficiency, nor did we find a significant difference in zinc levels between groups (P=0.36). In agreeement with this finding, Morshed et al. noted that serum zinc levels in thalassemia patients were within normal limits, so zinc supplementation was not needed.[24] Similarly, Kwan et al. reported that only 3 of 68 patients with thalassemia had zinc deficiency.[25]

We found higher interquartile range in thalassemia patients than in the control group, indicating more varied zinc levels in patients with thalassemia than the control group. Varied zinc levels may be due to variations in the age of subjects, the number of transfusions, and iron chelation therapy, or due to problems with anorexia, nutritional status, psychological problems, and different metabolic or endocrine complications. The highest zinc content was found in one thalassemia patient with 330.85 μg/dL. The patient at the time of the study was 3 years and 4 months old, with good nutritional and growth status, and 900 μg/dL ferritin level. This patient was routinely transfused every 4 weeks and never received iron chelation therapy, which may be a factor causing high zinc levels. High zinc levels in the thalassemia group compared to the control group were also reported in previous studies, possibly due to routine administration of blood transfusions, impaired zinc metabolism that occurs in patients with thalassemia, and a decrease in glomerular filtration rate.[26,27] A Tehran study examined zinc status in 64 people with beta thalassemia major compared to 64 healthy controls and obtained a significantly higher mean zinc level in thalassemia patients compared with the control group. They concluded that regular blood transfusions from healthy donors can prevent zinc deficiency.[19] A previous study in Jordan found a significant increase in zinc levels in the thalassemia group compared to the control group, due to a decrease in glomerular filtration rate of zinc and metabolic disorders of zinc that occur in thalassemia patients.[27]

Zinc concentration is higher in red blood cells. Patients who are dependent on transfusions have a higher serum zinc level than healthy controls. Kajanachumpol *et al.* reported an increase in erythrocyte zinc levels in thalassemia patients compared to healthy controls. The mechanism of increasing erythrocyte zinc is still unclear, but it is likely to be an effect of impaired zinc metabolism in the body which causes failure of zinc utilization in tissues of thalassemia patients.[28] The high serum zinc level in thalassemia patients is also associated with the possibility of liver parenchymal damage caused by hemosiderosis or a condition of decreased zinc glomerular filtration rate that occurs in chronic hemolysis.[19,27] The liver is a storage organ for zinc. Iron overload increases oxygen free radicals that induce peroxidative damage, increasing serum zinc from damaged hepatocytes. It has been hypothesized that variation in serum zinc level may be caused by endogenous leukocyte mediators that mobilize zinc from the liver and other tissues to the serum.[29] Metabolic zinc disorders are also a possible reason for high zinc levels in thalassemia patients.[27]

In patients with thalassemia, increased iron levels may be caused by recurrent blood transfusions and increased absorption due to ineffective erythropoesis.[14] Increased iron can inhibit the absorption of zinc in the gastrointestinal tract. There is a competitive inhibition between iron and zinc in binding to transferrin in the blood, while the administration of iron chelation in thalassemia patients would also chelate other important minerals including zinc.[30] Previous studies by Arijanty et al. and Nima et al. found that ferritin levels had a significant negative correlation with plasma zinc levels.[20,31] Similar results were also reported by Mahyar *et al.* in 2010 and Missiry *et al.* in 2014.[32,33] We noted a weak negative correlation between serum ferritin and zinc levels in thalassemia patients but it was not significant.

This study had several limitations, such as the small sample size. We also did not screen for a number of other clinical conditions that could bias the results of the study. The mechanism of increased zinc levels in thalassemia patients remains unclear and debatable. Further studies are necessary to determine factors that cause increased serum zinc levels in patients

with thalassemia.

In conclusion, there is no difference in serum zinc levels of pediatric thalassemia patients compared to healthy controls. In thalassemia patients, there is no correlation between ferritin levels and serum zinc levels.

Conflict of Interest

None declared.

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Original Article

UGT1A1 gene polymorphisms and jaundice in Indonesian neonates

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Abstract

Background Uridine diphospho-glucuronocyltransferase 1A1 (*UGT1A1*) polymorphisms are a risk factor for unconjugated hyperbilirubinemia in neonates. UGT1A1 polymorphisms decrease bilirubin conjugation, thus causing hyperbilirubinemia. A variety of polymorphisms have been reported, with *UGT1A1*60* and *UGT1A1*6* especially prominent in the Asian population. Hyperbilirubinemia polymorphism studies are lacking in Indonesian populations.

Objective To identify *UGT1A1*60* and *UGT1A1*6* profiles in Indonesian populations of heterogeneous ethnicity.

Methods We enrolled 42 jaundiced neonates who were born from January to April 2017 and treated in the Neonatal Intensive Care Unit of our national referral center, Cipto Mangunkusumo Hospital, Jakarta, Indonesia. Genetic mutations *60 of exon 1 and *6 of the promoter region were analyzed by polymerase chain reaction – restriction fragment length polymorphism methods, with DraI and AvaII as restriction enzymes, respectively. Clinical data including total serum bilirubin and racial information were obtained by medical records and interviews with parents.

Results There were no homozygous mutations of *UGT1A1*6*, but 4.8% of subjects were heterozygous. As for *UGT1A1*60*, 4.8% were heterozygous and 95.2% were homozygous. Racial variations were not observed for *UGT1A1*60*, while Betawi descendents were found to have many heteroygous forms of *UGT1A1*6*.

Conclusion Polymorphisms of the *UGT1A1* gene were found in Indonesian neonates. Some ethnicities also showed increased tendency towards its incidence, such as the heterozygous form of *UGT1A1*6*. **[Paediatr Indones. 2019;59:150-6; doi: http://dx.doi.org/10.14238/pi59.3.2019.150-6]**.

Keywords: *neonatal jaundice; PCR; polymorphism; RFLP; UGT1A1*

A physiological increase of bilirubin occurs in 60% of term and 80% of preterm neonates,[1] due to increased heme breakdown and physiological hepatic immaturity.[2] Hyperbilirubinemia can be observedseen on the skin and sclera as neonatal jaundice when the serum total bilirubin concentration reaches 5 mg/dL. Hyperbilirubinemia can lead to kernicterus spectrum disorder (KSD) when left untreated, especially in high risk babies. Kernicterus spectrum disorder is a neurologically debilitating condition that may lead to death.[3,4] Common risk factors for KSD are preterm birth, hemolytic disorders, and other delivery and postnatal conditions such as cephalohematoma, sepsis, and hypoalbuminemia.[4,5] Genetic factors have been studied extensively in past years. Caucasian and East Asian populations have been shown to have the *UGT1A1*28* polymorphism as a clinically significant risk factor for hyperbilirubinemia.[6,7]

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Bilirubin conjugation is catalyzed by the uridine diphospho (UDP)- glycosyltransferase (UGT) enzyme, which is encoded by the uridine diphospho-glucuronosyltransferase 1A1 (*UGT1A1*) gene.[8] Polymorphisms of *UGT1A1* polymorphisms decrease enzyme activity and lead to decreased bilirubin elimination, causing unconjugated hyperbilirubinemia conditions, such as Gilbert's syndrome.[9,10] One of the genetic variations involved in Gilbert's syndrome is the *UGT1A1*60* (c-3279T>G) polymorphism in the promoter region.11 This polymorphism has been demonstrated to contribute to the incidence of neonatal hyperbilirubinaemia in Malaysian and Taiwanese populations.[11,12] Another common polymorphism in East Asia is *UGT1A1*6* (c211G>A).[13] This polymorphism was found to be involved with development of hyperbilirubinemia in the Japanese population. However, in a study on Javanese-Indonesian and Malay-Malaysian populations, Sutomo *et al.* reported that *UGT1A1*6* was found, albeit in small frequencies and with no significant clinical correlation to the high incidence of hyperbilirubinemia.[14]

Information on variations of *UGT1A1* and the development of hyperbilirubinemia in Indonesia is still very limited. Racial variations are common in the diverse Indonesian population, but to date, only Javanese and Bengkulu populations have been studied.[1,15] As such, we aimed to obtain evidence and data on the *UGT1A1*6* and *UGT1A1*60* in the Indonesian population. The national referral hospital, Cipto Mangunkusumo Hospital, was chosen with the assumption that patients make-up may be roughly representive of the racial diversity of the Indonesian population.

Methods

This descriptive, cross-sectional study was conducted in neonatal patients with jaundice in the Neonatal Intensive Care Unit (NICU), Division of Perinatology, Department of Child Health, Cipto Mangunkusumo Hospital, Jakarta. Term and preterm neonates born from January to April 2017, as well as whose parents were of Indonesian descent and agreed to participate in the study, were included. Clinical jaundice was assessed by a neonatologist based on Kramer's index between day 3-7 post-natal. Neonates with hemolytic disease, showing clinical signs of sepsis, asphyxiation, and neonates who received blood transfusion or exchange transfusion, were excluded from the study. We used a total sampling method, where the number of samples taken was equal to the number of populations found during the duration of the study. Hence, of 42 jaundiced neonatal patients during the study period, all 42 were included in the study.

Clinical data including birth weight and total serum bilirubin levels were obtained from patients' medical records. Peak bBilirubin level was measured between the 3rd and 7th day of life. Ethnicity was obtained by ethnicity tracing from the parents with the aim of ascertaining their various ethnic or racial variations. Informed consent was obtained from both parents of the neonates. This study was approved by the Health Research Ethics Committee, University of Indonesia and the national referral hospital, Dr. Cipto Mangunkusumo Hospital, Jakarta.

Venous blood samples (1.5 mL) from neonates with jaundice in the NICU were collected into EDTA tubes and stored at -20°C. Genetic confirmation was done using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) analysis. Genomic DNA (g-DNA) was extracted either from fresh or frozen blood specimens using *QIAamp DNA Blood Mini Kit* (Qiagen, Germany).[15] Two microlitre

Table 1. Oligonucleotide primers used in this study

No	Oligonucleotide	Sequence	Target SNP	Variants
1	*UGT1A1*_promoter Fw	5'-CAC-CAGAACAAACTTCTGAG-3'	rs4124874? (c-3279T>G)	*UGT1A1*60*
2	*UGT1A1*_promoter Rv	5'-CTGTCCCTTCTG AAT-CATTG-3'	rs4124874? (c-3279T>G)	*UGT1A1*60*
3	*UGT1A1*_exon1 Fw /U1F1 forward	5'-AGATACTGT TGATCCCAGTG-3'	rs4148323 (c211G>A)	*UGT1A1*6*
4	*UGT1A1*_exon1 Rv /U211R reverse	5'-CTTCAAGGTGTAAAATGCTC-3'	rs4148323 (c211G>A)	*UGT1A1*6*

SNP: single nucleotide polymorphism

of g-DNA was added to a PCR master mix containing 29.3μL dH2O, 5μL 10X KOD Hot Start DNA polymerase buffer (Novagen, Germany), 4μl 25 mM MgSO4, 7μL 2 mM dNTPs (dATP, dCTP, dGTP, dTTP), and 0.7μL KOD Hot Start DNA polymerase (*Novagen*, Germany). Oligonucleotide primers used for this amplification are listed in **Table 1**.

The PCR amplification consisted of an initial denaturation for 2 min at 95°C, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55 °C for 1 min, post-extension at 72°C for 10 min, and held at 8°C for 60 min, in a PCR thermocycler TProfessional Standard 96 Gradient (*Biometra GmbH*, Germany). Amplicons were observed by 2% agarose gel electrophoresis in TAE 1X buffer containing ethidium bromide and visualized by UV transilluminator.

To determine the polymorphism at rs4148323 of *UGT1A1*6* by PCR-RFLP analysis, the restriction enzyme AvaII (*New England Biolabs*, USA) was used to digest the PCR amplicon of the exon 1 region of *UGT1A1* gene. Amplification was carried out by employing primer pairs for *UGT1A1* exon 1, as reported previously.[5]

To determine the polymorphism at rs4124874 of *UGT1A1*60* by PCR-RFLP analysis, the restriction enzyme DraI (*New England Biolabs*, USA) was used to digest the PCR amplicon of the promoter region of the *UGT1A1* gene. Amplification was carried out by employing primer pairs for the *UGT1A1* promoter, as reported previously.[11]

We used *STATA version 12* software for *MacOS* was used for statistical analysis. Only descriptive analysis was used for this study. Distribution of *UGT1A1* variants and racial profiles of the *UGT1A1* genotype were expressed with numbers and percentages.

Results

The PCR rs4124874 amplicon of *UGT1A1*60* was 141 bp, as shown by agarose gel electrophoresis in **Figure 1A**. The DraI restriction enzyme digestion resulted in two visible bands (**Figure 1B**). The homozygote type remained uncut, while the heterozygote resulted in three bands, i.e., 141 bp, 120 bp, and 21 bp, as reported by Huang *et al.*[11] However, using a 2% agarose gel, the small fragment of the amplicon cut by DraI could not be visualized.

The PCR rs4148323 amplicon of *UGT1A1*6* was 146 bp, as shown in **Figure 2A**. The AvaII restriction enzyme digestion resulted in two bands (**Figure 2B**). The homozygote remained uncut, while

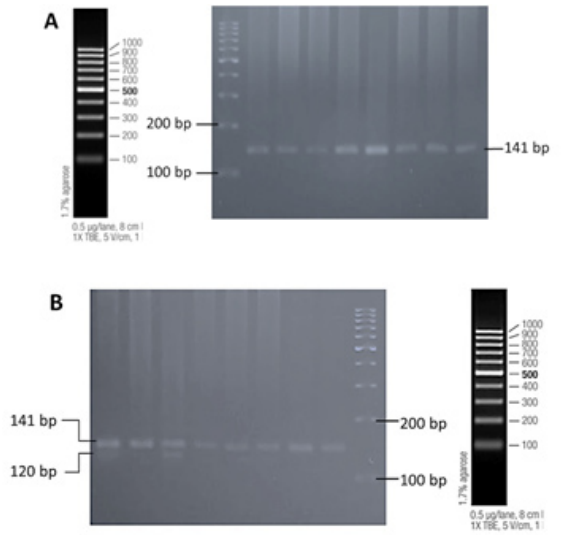

Figure 1. Agarose gel electrophoresis of (A) the PCR amplicon of *UGT1A1* rs4124874 (141 bp); and (B) the amplicon after being cut with DraI restriction enzyme (120 bp fragment, in addition to the 141 bp fragment; the 21 bp fragment could not be observed).

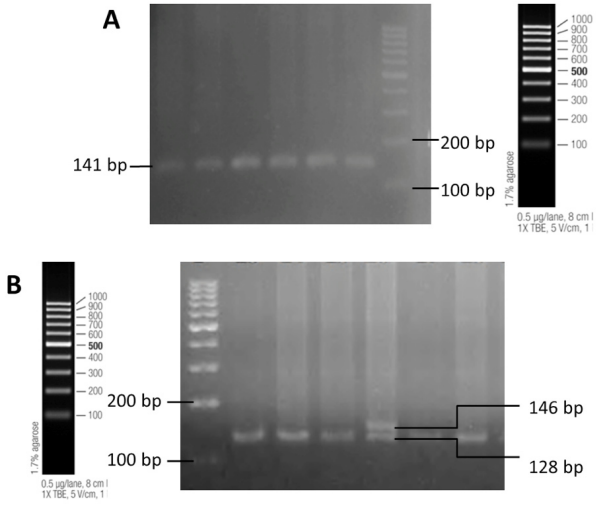

Figure 2. Agarose gel electrophoresis of (A) the PCR amplicon of *UGT1A1* rs 4148323 (146 bp); and (B) the amplicon after being cut with AvaII restriction enzyme (128 bp fragment, in addition to the 146 bp fragment; the 18 bp fragment could not be observed).

the heterozygote resulted in three bands, i.e., 146 bp, 128 bp, and 18 bp, as reported by Huang et al.5 However, using a 2% agarose gel, the small fragment of the amplicon cut by DraI could not be visualized.

Table 2 shows the distribution of *UGT1A1* variants of both the exon 1 *UGT1A1*6* mutation and the *UGT1A1*60* promoter mutation. Most of the exon 1 mutation (*UGT1A1*6*) did not occur, but all subjects had either homozygous or heterozygous promoter region mutation (*UGT1A1*60*). In our study, no subjects had the homozygous *UGT1A1*6* A/A genotype, only 4.8% of subjects had the heterozygous G/A genotype, and 95.2% of subjects had the G/G genotype (wild type) or did not have any SNP form of *UGT1A1*6*. With regards to *UGT1A1*60*, 95.2% had the homozygous G/G genotype, and 4.8% had the heterozygoous T/G genotype. No subjects had the wild type genotype (TT).

Table 3 shows that the *UGT1A1*6* heterozygous subjects (G/A genotype) came from parents of Betawi-Betawi and Betawi-Sundanese ethnicities. Whereas for *UGT1A1*60*, the T/T homozygous mutation, occurred in neonates of many ethnicities. In contrast with the *UGT1A1*6* variants, there was no specific ethnic tendency for the *UGT1A1*60* mutations based on our limited study.

Table 2. Distribution of UGT1A1 variants

Genotype	(N=42)
*UGT1A1*6*, n (%)	
G/G (wild type)	40 (95.2)
G/A (heterozygote)	2 (4.8)
A/A (homozygote)	0 (0)
*UGT1A1*60*, n (%)	
T/T (wild type)	0 (0)
T/G (heterozygote)	2 (4.8)
G/G (homozygote)	40 (95.2)

Discussion

Based on the detection of *UGT1A1* mutations in this study, as shown in **Table 2**, the *UGT1A1*6* mutations may not affect the incidence of hyperbilirubinaemia in Indonesian neonates. Similarly, Sutomo *et al.* showed that *UGT1A1*6* was rarely found in Javanese and Malaysian populations.[14] In our previous study of a Bengkulu population, interestingly we found over 60% of the neonates had SNP of *UGT1A1*6* in both healthy and jaundiced neonates.[15] This finding shows that even within Indonesian population, there exists an intra-ethnicity variation related with *UGT1A1*. The meta-analysis of *UGT1A1*6* studies in Southeast Asian populations including Malaysia and Thailand by Yu *et al.* showed the same results of 0% A/A genotype

Table 3. Genotypes of UGT1A1 polymorphisms by ethnicity in Indonesia

Ethnicity	UGT1A1*6 (n=42)			UGT1A1*60 (n=42)		
	A/A homozygous	G/A heterozygous	G/G wild type	T/T homozygous	T/G heterozygous	G/G wild type
Batak-Batakn, n (%)	0(0)	0(0)	1 (2.4)	1 (2.4)	0(0)	0(0)
Betawi-Betawi, n (%)	0(0)	1 (2.4)	6 (14.2)	8 (19)	0(0)	0(0)
Betawi-Sundanese, n (%)	0(0)	1 (2.4)	5 (11.9)	5 (11.9)	1 (2.4)	0(0)
Javanese-Javanese, n (%)	0(0)	0(0)	10 (23.8)	8 (19)	1 (2.4)	0(0)
Javanese-Lampung, n (%)	0(0)	0(0)	1 (2.4)	1 (2.4)	0(0)	0(0)
Javanese-Minangkabau, n (%)	0(0)	0(0)	1 (2.4)	1 (2.4)	0(0)	0(0)
Javanese-Sundanese, n (%)	0(0)	0(0)	3 (7.1)	3 (7.1)	0(0)	0(0)
Minangkabau - Minangkabau, n (%)	0(0)	0(0)	3 (7.1)	3(7.1)	0(0)	0(0)
Sundanese -Sundanese, n (%)	0(0)	0(0)	7 (16.7)	7(16.7)	0(0)	0(0)
Indonesian Chinese – Indonesian Chinese, n (%)	0(0)	0(0)	1 (2.4)	1 (2.4)	0(0)	0(0)
Bima-Bima, n (%)	0(0)	0(0)	1 (2.4)	1(2.4)	0(0)	0(0)
Javanese – Maluku, n (%)	0(0)	0(0)	1 (2.4)	1(2.4)	0(0)	0(0)

frequency, which differed from other Asian populations such as India, Japan, and China.[16] In certain conditions with co-existing risk factors, *UGT1A1*6* could still increase the risk for hyperbilirubinaemia. In one Japanese study, *UGT1A1*6* was shown to be a cause of prolonged unconjugated hyperbilirubinaemia.[17] In another Japanese study, *UGT1A1*6* was a risk factor for inadequate breastfeeding instead of breastmilk jaundice.[18] Therefore, despite the low incidence of *UGT1A1*6* in the Indonesian population, further study into multiple co-existing risk factors may prove *UGT1A1*6* to be a risk factor in Indonesia.

Due to the high incidence of *UGT1A1*60* in our study, this polymorphism could be a risk factor for the occurrence of unconjugated hyperbilirubinemia in Indonesian newborns. Yusoff *et al.* and Amandito *et al.* had similar results in Malaysian neonatal populations.[12,15] The Malaysian population is ethnically closer to the Indonesian population and located in Southeast Asia. Sutomo *et al.* found a similarity in *UGT1A1*60* mutation profiles in Javanese-Indonesian and Malay-Malaysian populations, which may be related to the anthropological proximity of Java-Indonesia and Malay-Malaysian populations.[14] In the population of neonates with hyperbilirubinemia in Malaysia, *UGT1A1*60* mutations play a role in causing hyperbilirubinemia.[12] However, Amandito *et al.* reported no significant correlation between the incidence of *UGT1A1*60* and total serum bilirubin level. This finding indicates that despite its high incidence, *UGT1A1*60* is not a clinically significant risk factor for hyperbilirubinemia.[15] In future studies, genetic mutations or other genes related to bilirubin conjugation or excretion should be included. It is also possible however, that clinical risk factors are more profound in the Indonesian population compared to genetic factors.

The incidence of mutations are related to ethnicity and race. Kanai *et al.* reported that the *UGT1A1*60* mutation was not significantly associated with neonatal hyperbilirubinemia in Japan.[19] Although the *UGT1A1*6* mutation did not contribute to the incidence of hyperbilirubinemia in the Javanese-Indonesian population, the *UGT1A1*6* frequency is high in the Japanese population. This may be due to the genetic differences between populations and other possible factors that may be significantly involved in the development of hyperbilirubinemia.[14] We can divide the Indonesian population into three major sub-racial groups: protomalay, deuteromalay, and melanesoid.[20] Despite the fact that both East Asian and Southeastern Asian are of Mongol racial descent, their sub-ethnicity differs from those of Malays, which could explain why the genetic mutation patterns differ.

In our study, there may be a tendency of the Betawi ethnicity to dominate the heterozygous mutation results, suggesting that ethnicity might influence *UGT1A1*6* incidences in our limited population (**Table 3**). This finding was in agreement with that of Zhang *et al.*, who noted an influence of intraethnic differences in certain ethnic groups on *UGT1A1* genetic variation.[21] The study was conducted on three Chinese sub-ethnics of Dong, Han, and She, which showed significant differences in genotypic frequencies between sub-ethnicities. The Han sub-ethnicity group carried the highest G/A genotype of *UGT1A1*6* frequency compared to sub-ethnicities of Dong and She.[21] In our study, Betawi ethnicity had the highest G/A genotype frequency compared to other ethnicities.

However, no ethnicity had a *UGT1A1*60* or is a wild type, as shown in **Table 3**. Therefore, the *UGT1A1*60* mutation in neonatal patients with unconjugated hyperbilirubinemia in Indonesia may not be influenced by a particular ethnicity or by racial diversity. In other racial groups, sub-ethnicity may affect the occurrence of a particular polymorphism, as noted by Zhang *et al.*[21] The frequency of *UGT1A1*60* was relatively higher in the Dong and Han ethnic groups than in the She group.[21]

Polymorphism *UGT1A1*60* may be a risk factor for neonatal hyperbilirubinemia in Indonesia, whereas *UGT1A1*6* may not affect the incidence of hyperbilirubinemia in neonates in Indonesia. The occurrence of mutations or gene polymorphisms may be related to ethnicity.

One limitation in our study is that we only included samples from one center and in limited number, therefore our sample is only a rough representation of the whole population in Indonesia. In addition, we did not include healthy neonates in our study as a control group, which limits the conclusion that we can take from our study. Discrepancies between ethnicities could also confound both the polymorphism and the bilirubin level. The method of

SNP analyses was also done through RFLP, whereas sequencing would have been superior in accuracy. We suggest conducting further study using a more accurate methods and a larger sample size with a more diverse population.

There is a high incidence of *UGT1A1*60* in Indonesian neonatal patients in Cipto Mangunkusumo Hospital, whereas the *UGT1A1*6* mutational incidence was very low. Despite all patients being of Indonesian descent, intra-ethnic differences in certain ethnicity groups may influence the genetic variation of *UGT1A1*6*, in which the Betawi ethnicity showed a small tendency of contributing more heterozygous SNP subjects. On the other hand, the *UGT1A1*60* mutation in neonates with unconjugated hyperbilirubinemia at Cipto Mangunkusumo Hospital is not affected by any particular ethnicity or racial diversity in Indonesia, as it occurred in all subjects. Further study is suggested to explore and confirm the genetic makeup of UGT1A1 and other related genes which could potentially contribute to a better understanding and treatment of neonatal jaundice of Indonesian neonates.

Conflict of Interest

None declared.

Acknowledgments

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Original Article

A five-year review of children with neuroblastoma at Dr. Sardjito General Hospital, Yogyakarta, Indonesia

Sutaryo, Scolastika Dita Kristian

Abstract

Background Neuroblastoma is the third most common tumor in children, after leukemia and retinoblastoma. The disease presents with a wide range of symptoms.

Objective To assess the clinical profiles of children with neuroblastoma at Dr. Sardjito General Hospital from 2012-2016.

Methods A retrospective review of all children with neuroblastoma under 18 years of age in the Children's Ward of Dr. Sardjito General Hospital, Yogyakarta from 2012-2016. Patients diagnosed and treated in other hospitals were excluded. Data were taken from the *Yogyakarta Pediatric Cancer Registry* (YPCR) and medical records. Outcomes were assessed by patient status: alive, died, or lost to follow-up.

Results A total of 40 subjects were included in this study. Six (15.0%) patients were diagnosed at <1 year of age, 26 (65.0%) patients at 1 to <5 years of age, 6 (15.0%) patients at 5 to <10 years of age, and 2 (5.0%) patients at ≥10 years of age. The male to female ratio was 1.5:1. Four (10.0%) patients had stage IV-S, 34 (85.0 %) patients had stage IV, and 2 (5.0 %) patients had stage II/III of the disease. Proptosis (40.0%) and abdominal mass (35.0%) were the most common chief complaints. Eight (20.0%) patients were alive at the end of observation, 15 (37.5%) died, and 17 (42.5%) were lost to follow-up. The deaths were mostly caused by sepsis.

Conclusion Most patients are diagnosed at the age of 1 to <5 years, with a median age of 3 years. Proptosis is the most common chief complaint. Most patients present in stage IV. Overall survival rate is very low. The high numbers of lost to follow-up should be noted. **[Paediatr Indones. 2019;59:157-63; doi: http://dx.doi.org/10.14238/pi59.3.2019.157-63]**.

Keywords: *neuroblastoma; pediatric; overall survival*

Neuroblastoma, an embryonic malignant tumor originating from the neural crest, is the third most common tumor in children after leukemia and retinoblastoma.[1] Each year, approximately 1,500 cases occur in Europe and 700 in the United States and Canada, accounting for about 28% of all cancers diagnosed in European and United States infants.[2,3] Its incidence peaks in infancy and then drops by half in the second year of life.[2] Cancer registries in developing countries are few and often insufficient for a number of reasons, not the least being lack of sustained funding and infrastructure, as well as absence of the recognition of cancer as a national health care priority.[3] A hospital-based registry, *Yogyakarta Pediatric Cancer Registry* (YPCR), has been ongoing in the Department of Child Health, Dr. Sardjito Hospital, Yogyakarta, Indonesia since 2000.1 There is a severe lack of information on pediatric cancer epidemiology in developing countries, which face such challenges as unreliable census data, under-reporting of cases, inaccurate diagnoses, and no

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certified documentation of deaths.[2] This study aimed to determine the clinical profiles of children diagnosed with neuroblastoma in Dr. Sardjito General Hospital from 2012-2016.

Methods

A retrospective review was conducted of children with neuroblastoma under 18 years of age in the Children's Ward of Dr. Sardjito General Hospital, Yogyakarta from 2012-2016. Patients diagnosed and treated in other hospitals and those with incomplete medical records were excluded. Data taken from the YPCR and medical records included age, sex, stage of disease, address, paternal education level, and clinical presentation. Diagnoses were based on diagnostic radiology test, biopsy, bone marrow aspiration, and laboratory tests, including vanillylmandelic acid (VMA), lactic dehydrogenase (LDH), and ferritin measurements. Risk stratification was assessed based on *International Society of Paediatric Oncology - Pediatric Oncology in Developing Countries* (SIOP-PODC) adapted risk stratification for low- and middle-income settings. Outcomes were assessed by patient status: alive, died, or lost to follow-up.

Descriptive data are presented in the text and tables. Patients were contacted by phone if they had not returned by June 30th 2017. Patients who could not be reached by phone were considered lost to follow up. Lost to follow-up patients were excluded from assessment of overall survival. Survival was measured from the date of diagnosis to the date of death or of the last follow-up appointment. Overall survival (OS) was estimated using Kaplan-Meier curves. Analyses were done using *SPSS version 25 for Windows* software.

Results

In total, 41 cases of neuroblastoma were identified from the hospital records between 2012 and 2016. One case was subsequently excluded due to the patient's diagnosis and treatment in another hospital. The disease incidence peaked in the 1-<5 years age group (**Table 1**), with a median age of 3 years at the time of diagnosis. Proptosis (40%) and abdominal enlargement (35%) were the most common chief complaints (**Table 2**). Most patients presented in stage IV (85%). All clinical presentations are shown in **Table 2**. Nine (22.5%) patients presented with bone metastases only, 8 (20.0%) patients with bone marrow metastases only, and 4 (10.0%) patients with bone, bone marrow, and intracranial metastases (**Table 3**). Thirty-four (85.0%) patients had primary tumors in the abdominal region, 26 of which arose from the adrenal medulla. Two primary tumors were found in the thorax and one in the pelvic region. There were three cases with unknown primary tumor region.

Table 1. Characteristics of subjects

Characteristics	(n=40)
Age at diagnosis, n (%)	
<1 year	6 (15.0)
1 to <5 years	26 (65.0)
5 to <10 years	6 (15.0)
≥10 years	2 (5.0)
Sex, n (%)	
Male	24 (60.0)
Female	16 (40.0)
Stage, n (%)	
I	0 (0)
II and III	2 (5.0)
IV	34 (85.0)
IV-S	4 (10.0)

Table 2. Clinical features

Clinical features	Number of patients
Chief complaints	
Protruding eye	16
Abdominal enlargement	14
Walking difficulties/ bone pain	7
Lump in waist	1
Pale	1
Testicular enlargement	1
Clinical presentation	
Pallor	38
Weakness	37
Loss of appetite	34
Abdominal mass	27
Hepatomegaly	25
Lymphadenopathy	26
Raccoon eye	23
Fever	23
Splenomegaly	16
Head lump	15
Walking difficulties	15
Bleeding	10
Pleural effusion	9
Others	3

Twenty-three (57.5%) patients underwent biopsy, and only 12.5% underwent immunohistochemistry (IHC) examination. The VMA, LDH, and ferritin examinations were done in less than 50% of patients (**Table 4**).

Chemotherapy regimens consisted of vincristine 1.5 mg/m^2 and cyclophosphamide 600 mg/m^2 on first day, cisplatin 80 mg/m^2 on the second day, and etoposide 200 mg/m^2 on the third day. Chemotherapy was given for 8 cycles, with a 3-week interval between each cycle. Fifteen (37.5%) patients were died at the end of the observation and 17 (42.5%) were lost to follow-up. The deaths were mostly caused by sepsis (6 cases) (**Table 5**). Most of the lost to follow-up patients did not return to continue treatment. Most of patients came from other provinces (60.0%) (Table 1) and lacked communication access, so it was difficult to trace them.

Table 3. Metastases sites

Site	(N=40)
Bone	9 (22.5)
Bone marrow	8 (20.0)
Bone and bone marrow	5 (12.5)
Bone marrow, intracranial, and bone	4 (10.0)
Bone and intracranial	2 (5.0)
Bone marrow and intracranial	1 (2.5)

Table 4. Diagnostic procedures

Type of diagnostic procedure	(N=40)
Biopsy	
Yes	23 (57.5)
No	17 (42.5)
IHC	
Yes	5 (12.5)
No	35 (87.5)
Bone marrow aspiration	
Positive	18 (45.0)
Negative	17 (42.5)
No	5 (12.5)
VMA level	
≤8 mg/24 h	15 (37.5)
>8 mg/24 h	1 (2.5)
No	24 (60.0)
Ferritin level	0 (0)
<120 ng/mL	5 (12.5)
≥120 ng/mL	35 (87.5)
No	
LDH level	
<750 IU/mL	2 (5.0)
≥750 IU/mL	7 (17.5)
No	31 (77.5)

Table 5. Cause of death

Cause of death	Number of patients
Sepsis	6
Intracranial metastases	2
Pulmonary metastases	2
Pulmonary edema	2
Abdominal compartment syndrome	1
Acute respiratory distress	2

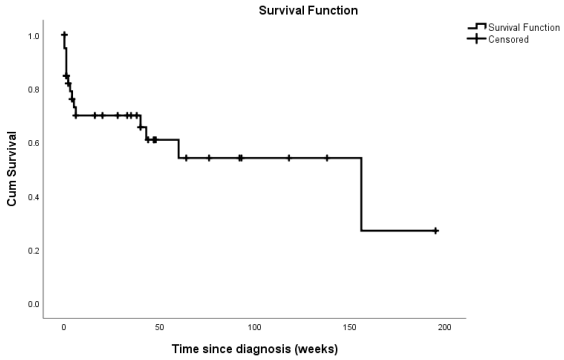

Figure 1. Overall survival

Survival analysis was carried out on 40 patients. Our overall survival was 27.1% (**Figure 1**). It demonstrated that the survival rate was low, especially in our hospital from 2011-2016.

Discussion

Neuroblastoma represents 5.5% of all malignant diseases in children in the Department of Child Health, Faculty of Medicine Universitas Gadjah Mada/Dr. Sardjito General Hospital, Yogyakarta.[1] Neuroblastoma is the third most common type of cancer in children, after leukemia and retinoblastoma.[1,4,5]

Previous reports indicate that 40% of neuroblastoma cases occur in the first year of life, 35% between the ages of 1 to 2 years, and 25% after the age of 2 years.[6,7] In our study, 15.0% of cases occurred at <1 year of age. The percentage of patients with neuroblastoma in the 1 to <5 year age group was most prevalent (65%), compared to other age groups. In a previous study, the median age at diagnosis was 2 years and 90% of cases were diagnosed before the age

of 5 years.[8] The median age diagnosed in our study was 3 years. Neuroblastoma is more common in boys than girls with a ratio of 1.1: 1 to 1.5: 1.9.9,10 Our study had a male: female ratio of 1.5:1.

Protruding eye and abdominal distension were common chief complaints, involving 16 (40.0%) and 14 (35.0%) cases, respectively. Walking difficulties or bone pain were observed in 7 (17.5%) cases, indicating the presence of bone metastases or spinal compression.[11,12] Pallor and weakness were also common accompanying symptoms, with 38 (95.0%) and 37 (92.5%) cases, respectively, followed by decreasing appetite in 34 (85.0%) and abdominal mass in 27 (67.5%) cases. Bansal *et al.* reported 103 cases with neuroblastoma in India and found fever in 67 (65%) cases, abdominal distension or abdominal pain in 56 (54.4%) cases, bone pain in 32 (31%) cases, proptosis in 28 (27.2%) cases, paresis of lower extremity and urinary incontinence in 2 cases each, jaundice in 3 cases, and chronic diarrhea, hematuria, abnormal gait, and abnormal eye movement in one case each. On physical examination, they found hepatomegaly in 31 cases (30%), bone lesion in 26 (25.5%), splenomegaly in 15 (14.6%), lymphadenopathy in 14 (13.6%), central nervous system metastases in 7 (6.8 %), bilateral pleural effusions in 4 (3.9%), and superior vena cava syndrome with opsomyoclonus and ataxia in one case.[13] In our study, there were no cases of opsomyoclonus, ataxia, or superior vena cava syndrome. Opsomyoclonus and ataxia are often associated with good prognoses.[14,15]

Instead of history-taking and physical examination, the diagnosis of neuroblastoma is based on either small round blue cells in the tumor biopsy or by identification of rosette cells in the bone marrow.[16,17] Urine catecholamine test is recommended.[18] Biopsy can be done in the primary tumor or sites of metastases.[19] In our study, biopsy was done in 23 (57.5%) cases; the rest were determined based on examination of bone marrow aspiration and/or CT scan. Immunohistochemistry staining that may be used includes neuron-specific enolase, tyrosine hydroxylase, CD-56, and synaptophysin.[20,21] In our study, only 5 patients had immunohistochemistry staining and 16 (40.0%) had VMA examinations. Of these 16 patients, only 1 patient had an abnormally high level. The sensitivity of VMA was reported to be 80.7%, but increased to 91.2% when combined with HVA.[22] The levels of VMA and HVA are considered to be high if above 2.5 SD based on levels in healthy children of an age-matched.[23]

Most primary tumor sites were found in the adrenal medulla (61.5%). The rest were unknown. Metaiodobenzylguanidine (MIBG) scintigraphy has high sensitivity and specificity (88% and 99%, respectively) in defining tumor sites, both primary and recurrent.[19,24] The MIBG scintigraphy is also recommended for metastases of disease.15 However, this modality is currently unavailable in our hospital.

In our study, 85.0% patients presented with stage IV, a late stage of disease. Unspecific signs and symptoms are some of the causes for late presentation.[11] Bone metastases were found in 45.0% of our patients. Metastases are mostly found in long bones and skull, bone marrow, liver, lymph nodes, and skin.[11,25] Pulmonary and intracranial metastases are rarely seen, although there is often hematogenous spreading.[8] We found pulmonary metastases in 2 (5%) cases and both of them died. Of 7 patients with intracranial metastases, 4 died and the others were lost to follow-up.

Risk stratification is defined by Shimada histology, MYCN, *International Neuroblastoma Pathologic Classification* (INPC) classification, and 11q aberration.[26] Most developing countries do not have these standard examinations. As a result, in 2015, *International Society of Paediatric Oncology - Pediatric Oncology in Developing Countries* (SIOP-PODC) created risk stratification guidelines for low-middle income countries with limited resources. That classification uses *International Neuroblastoma Staging System* (INSS), initial status, age, LDH level, ferritin level, and MYCN status (if known).[16] In our study, 33 cases were high risk.

Increasing LDH is a strong prognostic indicator of poor outcome and correlates with unfavorable histology.[26,27] Patients with LDH >1,300 IU/mL have 12.9 times greater risk for relapse.[28] *International Neuroblastoma Risk Group* (INRG) found that LDH level more than 587 IU/mL has poor prognostic. High level of ferritin correlates with low event-free survival (EFS), but positively associated with stage.[20] The LDH and ferritin levels are not included in the INRG stratification because of lack of specificity. The SIOP-PODC risk stratification using LDH and ferritin

levels with thresholds of 750 IU/mL and 120 ng/mL, respectively.[16]

The patients with the worst prognoses are children aged >15 months, those at an advanced stage, and those testing positive for several molecular biology markers such as MYCN. The V-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN) is an oncogene for regulating cell proliferation and apoptosis.[29,30] The MYCN is positive in 5-10% cases in children under 12 months and 20-30% cases in later ages.[2] Overall survival in developed countries has increased, along with the use of intensive therapy.[31,32] Five-year overall survival in the United States and Europe is 58%, but only 10% in developing countries.[33,34] In our hospital from 2000 to 2004, the OS was 20%.[35] Our study revealed overall survival rate was 27.1%. Fifteen (37.5%) patients were died at the end of the study and 17 (42.5%) were lost to follow up. The deaths were mostly caused by sepsis (6 cases).

Of the 15 patients who died, four did not receive therapy. They presented with abdominal distention, bone metastases, intracranial metastases, and pulmonary metastases. Seven patients died in the first cycle of chemotherapy. They also presented with multiple site metastases. Patients with poor clinical condition could not tolerate the side effects of chemotherapy agents. Palliative chemotherapy should be used in these cases.[15] Four patients died after receiving complete chemotherapy. Three patients had progressive disease and intracranial metastases. One patient had no response to treatment and died because of sepsis.

The cause of loss to follow up was patients not returning to continue therapy. It was difficult to trace these patients' latest conditions because of lack of communication access and distant home addresses. Twenty-four (60.0%) patients lived in the outer areas of Yogyakarta Province, as Dr. Sardjito General Hospital is a referral hospital from all parts of the province. Communication access was difficult because the phone numbers provided were not active and some were phone numbers of village officials who had accompanied the patients.

In conclusion, neuroblastoma is one of the most common tumors in children. Most patients are diagnosed at 1-<5 years, with a median of 3 years. Proptosis is the most common chief complaint. Most patients (85.0%) present in stage IV. Overall survival is very low. The high number of patients lost to follow up should be noted.

Conflict of Interest

None declared.

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Case Report

Management of pink tetralogy of Fallot in VACTERL association

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Tetralogy of Fallot (TOF) and vertebral abnormalities, anorectal malformation, cardiac defects, tracheoesophageal fistula with or without esophageal atresia, renal malformations, and limb abnormalities (VACTERL) are considered rare entities requiring medical attention with regards to diagnosis and management. When ToF is associated with VACTERL association, case management might differ from a simple case of ToF.

Tetralogy of Fallot (ToF) is defined as a complex congenital cardiac abnormality characterized by ventricular septal defect, right outflow tract obstruction, right ventricular hypertrophy, and overriding aorta.[1] The term was coined in 1888 by Fallot of Marseilles, even though the defect had been described in 1673 by Steno of Denmark. The entity was then studied extensively to deliver better care management through medical and surgical approaches.[2] **[Paediatr Indones. 2019;59:164-8; doi: http://dx.doi.org/10.14238/pi59.3.2019.164-8]**.

Keywords: *pink TOF; VACTERL association; ACE inhibitor; congenital solitary kidney*

Tetralogy of Fallot is considered the most prevalent cyanotic heart disease, comprising 10% of all congenital heart disease and occurring in three to six infants for every 10,000 births.[3] Among infants with ToF, acyanotic ToF or pink ToF is considered a small minority on the clinical spectrum of infants with ToF. Pink ToF is characterized by mild pulmonary stenosis and small left-to-right shunt in the ventricular septal defect.[4] However, to our knowledge, its prevalence has yet to be studied. Pink ToF itself is a rare entity, but being a part of VACTERL association is even rarer.[5] However, ToF is a common cardiac manifestation of VACTERL, with around 75% of VACTERL association cases having ToF.[6]

Standard management of ToF consists of early recognition, medical therapy, and surgery, divided into either staged palliation or a primary repair approach.[7] In the neonatal period, ToF may not manifest as a full-blown disease when presenting right after birth, as right ventricular failure may not be apparent. But as the condition progresses, right ventricular hypertrophy may develop and lead to right heart failure, if pressure reduction and anti-myocardial remodeling therapy is not given.[8] Standard ToF therapy, as mentioned above, may not be applicable in cases with other associated

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organ anomalies such as VACTERL association, further imposing a challenge to deliver optimal therapy to prevent long-term complications of ToF.

Therefore, the purpose of reporting this case was to emphasize the importance of early recognition for congenital heart disease that may be related to other organ anomalies, to report a method which can be applied in medical facilities with limited resources, and lastly, to define the best possible treatment based on scientific evidence in order to reduce or prevent long-term complications of ToF.

The Case

A three-day-old female neonate with cloacal-type anal atresia (**Figure 1**) was referred from a district hospital. She required a colostomy, but no other diagnosis was mentioned in the referral. The baby was delivered vaginally at 35 weeks gestation, with APGAR score 2/3/8 (at minute one, five, and ten), and birth weight of 2,100 gr. The mother denied consuming any alcohol, smoking, or experiencing any period of illness during the gestation period.

Upon examination in the referred hospital, a holosystolic grade 4/6 murmur best heard at the left lower sternal border was noticed, but with no central or peripheral cyanosis presentation. Echocardiography was done to confirm the diagnosis of acyanotic congenital heart defect. The heart was found to be situs solitus, with apparent subaortic ventricular septal defect (VSD), less than 30% overriding aorta, and infundibular region pulmonary valve stenosis, based on transthoracic echocardiography (TTE). The patient was then diagnosed with non-cyanotic ToF or pink ToF.

After finding anal atresia and pink ToF, the patient was further examined for other related anomalies. Head ultrasound showed no malformation, but abdominal ultrasound revealed right renal agenesis and left hydronephrosis. Chest x-ray revealed butterfly vertebrae at Th5 and Th9, as well as unilateral-unsegmented-bar sacral vertebrae (**Figure 2**). The child's feet were found to be internally rotated with straightened Achilles tendon, signifying the diagnosis of congenital talipes equinovarus (**Figure 3**). Based on the aforementioned anomalies, the patient was diagnosed with VACTERL association.

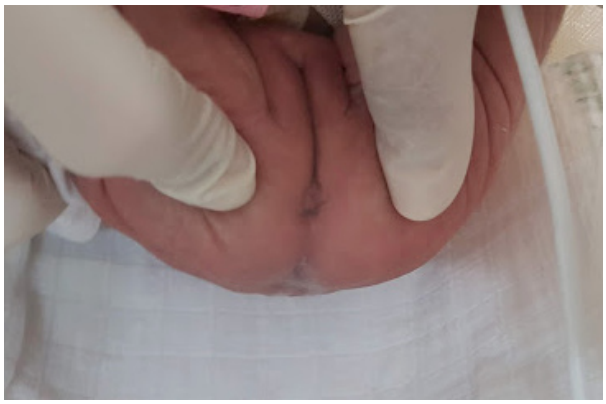

Figure 1.Anal atresia presented as cloaca

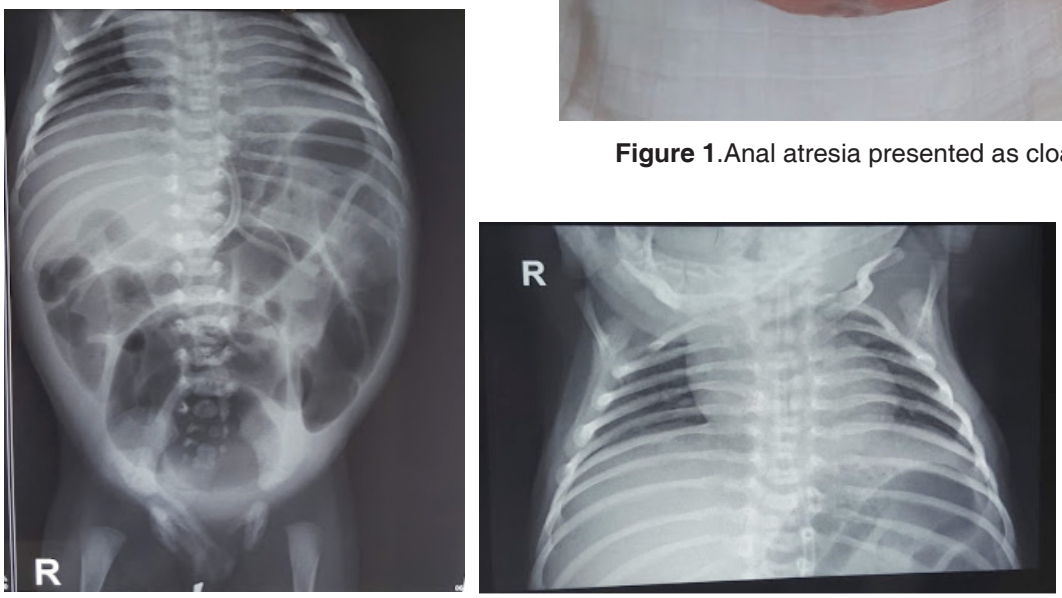

Figure 2. Butterfly vertebrae in Th5 and Th9 with unilateral-unsegmented-bar sacrum

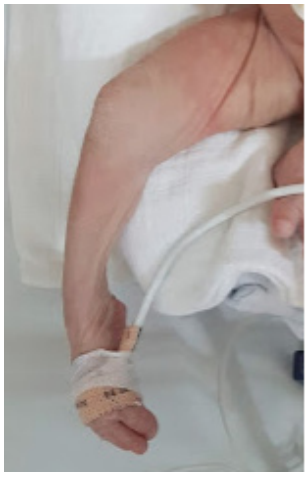

Figure 3. The patient's foot position was found to be internally rotated with straightened Achilles tendon (talipes equinovarus)

The patient underwent surgical colostomy as management for anal atresia. However, no surgical approach was planned for the other organ anomalies. With regards to pink ToF, a surgical approach was also withheld due to the clinical condition of the patient. The neonate would undergo watchful waiting and be re-evaluated in one month. Administration of angiotensin convertase (ACE) inhibitor was also withheld, due to the risk of renal failure since the infant had a congenital solitary kidney associated with VACTERL association.

MRI and right heart catheterization was prepared for further follow-up management plan. If the patient meets the criteria for ToF correction, the procedure of ToF correction would be done in the fourth month of life.

Discussion

The VACTERL association is considered to be a rare congenital disease with an incidence ranging from 1 in 10,000 to 1 in 40,000 live births,[9] which includes vertebral anomalies (V), anal atresia (A), cardiac malformation (C), tracheo-oesophageal fistula (TE) with or without esophageal atresia, renal dysplasia (R), and limb abnormalities (L). The entity was first mentioned as VATER association in 1973 and considered as multiple organ anomalies without evidence of a single unifying cause.[10] However, the likelihood of "developmental field defect" occurring in the blastogenesis phase has been speculated.[5]

A VACTERL association diagnosis requires the presence of at least three congenital malformations. In our case, the malformations were vertebral abnormalities (butterfly vertebrae and unilateral-unsegmented-bar sacrum), anorectal malformation (cloacal-type anal atresia), cardiac defects (pink ToF), renal abnormalities (right renal agenesis), and limb abnormality (congenital talipes equinovarus).[5]

Infants with congenital heart disease (CHD) may benefit from early screening as it may reduce morbidity and mortality from early intervention.[11] Early detection of CHD may be as early as in the prenatal period using fetal echocardiography screening.[12] However, the procedure cannot be implemented widely at the present time in Indonesia since there are a limited number of experts in fetal echocardiography. As such, screening neonates right after birth may also be done in medical centers with limited resources using oximetry screening. This tool measures oxygen saturation at the pre-ductal (right hand) and post-ductal (foot) levels, and may be useful for screening for possible CHD. This simple approach is more sensitive and specific (76.5% and 99.9%, respectively) to screen for CHD, rather than depending only on a cyanotic appearance of the infant.[13]

The short-, middle-, and long-term complications of ToF management should be considered in advance. Short-term management might be related to overcoming the hypoxic condition indicated by a cyanotic appearance. Middle- and long-term complications may be associated with heart failure and multi-organ diseases.[8] The pulmonary stenosis in ToF can be considered as RVOT if it results in cyanosis. This short-term complication of RVOT is influenced by the degree of RVOT obstruction and hypoplastic pulmonary valve annulus as the main culprit of the hypoxic condition.[14] In addition, middle- and long-term complications of TOF are endocarditis, cerebral infarction or abscess, right ventricular hypertrophy with right heart failure, or progressive hypoxia as the disease progresses. These entities will in turn be the major causes of morbidity and mortality of TOF patients. Implementing primary repair of the defect through a surgical approach is the best prevention.[15]

Standard medical management of ToF is especially needed if pulmonary atresia (ductal-dependent ToF) is present. Infusion of prostaglandins (initial: 0.05-0.1 mcg/kg/min IV; maintenance: 0.01-0.4 mcg/kg/min) is needed to ensure the patency of ductus arteriosus.[16] Alternatively, surgical management of ToF has developed significantly, shifting to an earlier age. Symptomatic ToF is categorized as ductal-dependent pulmonary circulation needing prostaglandins to open the ductus arteriosus and has been associated with less than 75% of oxygen saturation. Urgent primary surgical repair or Blalock-Taussig shunt is indicated for these patients.[14] However, for elective procedures, a two-stage surgery or primary repair approach has been proposed for as early as the neonatal period.[15] The size of the pulmonary artery branches should be considered when selecting between the palliative shunt approach and the primary repair approach.[17] A retrospective study in 1992 reported that primary repair of ToF in young infants less than three months of age was associated with high mortality.[18] However, with improved techniques, early primary repair has been associated with better outcomes, such as avoiding shunt-related complications, early relief of hypoxia, promotion of normal lung development, and avoidance of right ventricular hypertrophy.[19,20]

Our patient presented with a non-cyanotic appearance, consistent with a diagnosis of pink ToF. Therefore, she did not need prostaglandin therapy. Nonetheless, a medical approach which functions as anti-remodeling therapy is also noteworthy. Angiotensin convertase (ACE) inhibitor is considered as the cornerstone therapy to try to deter the progression of right ventricular hypertrophy related to pressure and volume overload.[21,22] However, ACE inhibitor administration was postponed in our patient, considering that she had a congenital solitary kidney as part of VACTERL association. Contrary to the belief that ACE inhibitors may lead to kidney damage, Simeoni M et al. showed that anti-renin angiotensin-aldosterone system drugs were renoprotective in patients with a solitary kidney.[23] As such, we considered the use of ACE inhibitors in our case, at an accordingly adjusted dose, but we ended up not doing so. Alongside the medical management of this child, the team concluded that watchful waiting for the need of surgical repair might be a safe path. Moreover, there was no urgent benefit to short-term management of complications of pink ToF. However, considering the possibility of worsening right ventricular function in the future, an earlier primary surgical repair approach should be considered as a rational step. Pozzi M *et al.* also mentioned that asymptomatic pink ToF might benefit the most from early repair.[15]

The ideal management of congenital heart disease starts with prenatal screening using fetal echocardiography, which is still not widely applicable in Indonesia. However, a simple approach such as pre-ductal and post-ductal oxygen saturation screening might significantly improve detection of congenital heart disease, especially in remote areas or hospitals with limited medical resources. Since early screening lead to early disease management, our patient with pink ToF and congenital solitary kidney, which are the part of VACTERL association, might have benefited from strictly adjusted ACE inhibitor administration and early primary surgical repair.

Conflict of Interest

None declared.

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Original Article

Predictive factors of ketoacidosis in type 1 diabetes mellitus

Listianingrum, Suryono Yudha Patria, Tunjung Wibowo

Abstract

Background Diabetic ketoacidosis (DKA) is an acute complication in type 1 diabetes mellitus (DM) and a significant cause of morbidity and mortality in developing countries. Diabetic ketoacidosis can be prevented by good management of the disease. Risk factors from previous studies that increase the DKA incidence were p eripubertal age, female gender, low socio-economic status, low parental education status, DKA at the first diagnosis of type 1 DM, infection, psychological problems, poor metabolic control, and non-compliance with insulin treatment.

Objective To determine whether sex, age, socio-economic status, parental education level, DKA at the initial diagnosis of type 1 DM, infection, psychological problems, poor metabolic control, and failing to take insulin as needed were predictive of DKA in type 1 DM patients.

Methods We conducted a retrospective cohort study using medical records from type 1 DM patients aged 0-20 years, at the Department of Child Health, Dr. Sardjito Hospital, Yogyakarta, from January 2011 to May 2017. We assessed for the occurrence and predictors of DKA. Logistic regression analysis was done to determine which factors increased DKA incidence.

Results A total of 57 type 1 DM patients were recruited, with DKA incidence of 37 (65%). Five (8.8%) DKA patients died. Multivariate analysis revealed that infection (OR 5.23; 95%CI 1.47 to 19.68; P=0.014) and DKA at the first diagnosis of type 1 DM (OR 5.37; 95%CI 1.40 to 19.52; P=0.011) were significant risk factors for DKA.

Conclusion Infection and DKA at the first diagnosis of type 1 DM are significant predictors of increased DKA incidence. **[Paediatr Indones. 2019;59:169-74; doi: http://dx.doi.org/10.14238/pi59.4.2019.169-74]**.

Keywords: *ketoacidosis; type-1 diabetes mellitus; predictor factor*

Diabetic ketoacidosis (DKA) is an acute complication of type 1 diabetes mellitus. This condition causes significant mortality and morbidity in type 1 DM patients. Mortality due to DKA in developed and developing countries is around 0.15-0.31% and 3.4-13.4%, respectively.[1,2] The social and economic burden inflicted by DKA is large, especially with regards to hospitalization. In the US, over 62% of hospital care in type 1 DM patients is associated with DKA incidence. The cost of type 1 DM treatment in hospitals doubles if complicated by DKA.[3]

Incidence rates of DKA vary and are influenced by geographic, socio-economic and health care facility conditions in each area. The prevalence of DKA varies considerably in many countries, ranging from 13-80%, with higher prevalence in developing countries than in developed countries. The incidence of DKA in children diagnosed with type 1 DM is 1-10% per patient per year.[1,4] In Indonesia, no exact data for incidence rate

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of DKA is available, but a Jakarta study in 2007 reported a prevalence of DKA in type 1 DM of 76.9%.[5] Based on medical record data in Dr. Sardjito General Hospital, Yogyakarta, the prevalence of DKA during 2014-2016 was 62.5%. Previous studies reported that the risk of DKA increases in children with poor metabolic control, history of DKA at first diagnosis, non-compliance with insulin treatment, infection, peripubertal age, and emotional/psychological problems, including eating disorders.[6,7,8]

Proper treatment of ketoacidosis generally results in good outcomes. The success of DKA management depends on initial identification of its signs and symptoms, especially in correcting dehydration, acidosis, electrolyte imbalance disorder, and hyperglycemia.[9] Diabetic ketoacidosis can be prevented by controlling the risk factors that trigger DKA. Predictors of DKA in a particular region should be identified to determine modifiable factors, in order to prevent DKA and lower its morbidity and mortality. This study aimed to identify characteristics of patients with DKA and predictive factors that affect the occurrence of DKA.

Methods

This study was a retrospective cohort study by examining medical record data at Dr. Sardjito General Hospital, Yogyakarta, from January 2011 - May 2017. The inclusion criteria were patients who had been diagnosed with type 1 DM, aged 0 days to ≤ 20 years, and treated at Dr. Sardjito General Hospital in the study period. Exclusion criteria were incomplete medical record data (i.e., missing two or more variables), overweight or obesity, using drugs that affect blood sugar levels (glucocorticoids, thyroid hormones, diazoxides, thiazides, or dilantin), or normal or high C-peptide level. The dependent variable was the occurrence of DKA. The independent variables were DKA upon diagnosis of type 1 DM, age, compliance to insulin treatment, infection, metabolic control, psychological problems, biological sex, parental education level, and socio-economic level. Compliance with insulin treatment was defined as a patient compliance to use appropriate insulin with recommended dose, not-compliance with insulin treatment was defined as a patient which use insulin not appropriate more than one day from recommended dose. Infection was defined as urinary, digestive, or respiratory tract infection that occurred before DKA, diagnosed from anamnesis, physical and laboratory examination in first 24 hours at hospital. Preschool was defined as 0-8 years of patient age, and peripubertal was 8-20 years of patient age. Parental education level was defined as a last formal educational state of the parental patient that from elementary until junior high school we categorized as low parental educational level, senior high school we categorized as middle parental educational level and diploma, university, magister and doctor we we categorized as high parental educational level. We assest metabolic control based on HbA1c level three month before until three month after DKA. Socio-economic level was graded based on family income; we categorized low socio-economic level if the family income below Rp 1.250.000, middle socio-economic level if the family income Rp 1.250.000 – Rp 5.000.000 , and high socio-economic level if the family income more than Rp 5.000.000.

Data were collected from medical records by filling out questionnaires by the researcher. The relationship between independent variables and the dependent variable was analyzed by bivariate statistical analysis (Chi-square test). From the bivariate analysis, variables with P values <0.25 were further analyzed by multivariative backward logistic regression method. This study was approved by the Medical Ethics Committee at Universitas Gadjah Mada.

Results

There were 57 cases (eligible subjek) of type 1 DM in Dr. Sardjito General Hospital during January 2011-May 2017. The basic characteristics of subjects are shown in **Table 1**.

The occurence of DKA was 65% (37/57). The signs and symptoms included shock (54.1%), nausea and vomiting (89.2%), abdominal pain (73%), Kussmaul breathing (86.5%), and decreased consciousness (59.5%). Mean (SD) laboratory results in the event of DKA were as follows: pH 7.10 (SD 0.12); HCO3 6.71 (SD 5.21) mEq/L; base excess (BE) -21.58 (SD 6.33); corrected sodium 141.51 (SD 8.07) mmol/L; potassium 4.32 (SD 1.07) mmol/L; blood glucose 509.3 (SD 151.6) g/dL, and positive ketonuria 3.4 (SD 0.6) mmol/L. A total of 10 (27%)

Table 1. Basic characteristics of subjects

Characteristics	Total (n=57)
Sex, n (%)	
Female	40 (70.2)
Male	17 (29.8)
Age, n (%)	
Infant to pre-school (0-8 year)	8 (14)
Peripubertal (8-20 year)	49 (86)
Parental education level, n (%)	
Low	24 (42.1)
Middle	17 (29.8)
High	16 (28.1)
Socio-economic level, n (%)	
Low	16 (28.1)
Middle	18 (31.6)
High	23 (40.4)
DKA at the initial diagnosis of type 1 DM, n (%)	
Yes	34 (59.6)
No	23 (40.4)
Mean duration of type 1 DM (SD), years	3.93 (3.00)
Nutritional status, n (%)	
Normal	37 (64.9)
Moderate & severe malnutrition	20 (35.1)
Infection, n (%)	
Yes	31 (54.4)
No	26 (45.6)
Kind of infection, n (%)	
Urinary tract infection	12 (38.7)
Respiratory infection	12 (38.7)
Gastrointestinal infection	4 (12.9)
Sepsis	3 (9.7)
Metabolic control, n (%)	
Poor	51 (89.5)
Moderate	4 (7)
Good	2 (3.4)
Mean HbA1C level (SD), %	11.12 (2.71)
Insulin therapy non-compliance, n (%)	
Yes	13 (22.8)
No	44 (77.2)
Psychological problems, n (%)	
Yes	18 (31.6)
No	32 (51.6)
No data	7 (12.3)
Mortality, n (%)	
Died	5 (8.8)

subjects with DKA required intensive care in the PICU. Mean (SD) length of treatment per episode of DKA was 8.17 (SD 6.21) days. The mortality rate from DKA was 5/37. The outcomes of DKA patients are summarized in **Table 2**.

Bivariate analysis revealed that infected subjects (OR 7.09; 95%CI 2.07 to 24.34; P=0.001) and DKA at the first diagnosis of type 1 DM (OR 7.26; 95%CI 2.15 to 24.49; P=0.01) had significantly different proportions for DKA occurence compared to those who had no infection or had no DKA at the first diagnosis of type 1 DM. The bivariate analysis results are presented in **Table 3**.

Table 2. Subjects' laboratory and clinical profiles as well as DKA outcomes

Clinical and laboratory profiles	Total subjects (n=37)
Clinical symptoms, n	
Shock	20
Nausea-vomiting	33
Abdominal pain	27
Kussmaul breathing	32
Decreased consciousness	22
Laboratory profiles	
Mean pH (SD)	7.10 (0.12)
Mean HCO3 (SD), mEq/L	6.71 (5.21)
Mean base excess (SD)	-21.58 (6.33)
Mean blood glucose level (SD), g/dL	509.3 (151.6)
Mean ketonuria (+) (SD), mmol/L	3.4 (0.6)
Mean corrected sodium (SD), mmol/L	141.51 (8.07)
Mean potassium (SD), mmol/L	4.32 (1.07)
Mean chloride (SD), mmol/L	102.44 (8,63)
Mean hemoglobin (SD), g/dL	13.63 (1.72)
Mean leukocyte count (SD), x10^3/mm^3	22.80 (11.92)
Hospitalization in PICU, n	10 (27)
Mean duration of hospitatization per DKA episode, days (SD)	8.17 (6.21)
Severity of DKA, n	
Mild	7
Moderate	10
Severe	20

To analyze the relationships between independent variables (predictors) and DKA, we performed a multivariate analysis. All variables with P value <0.25 from the bivariate analysis were included in the multivariate backward logistic regression analysis. The first step result of analysis is presented in **Table 4**.

Initial stage multivariate analysis revealed that psychological problems, poor metabolic control, insulin non-compliance, and female sex to have P values >0.05, hence, they were eliminated from the next stage of analysis. The previously uneliminated variables were analyzed using logistic regression. Any variable with P >0.05 could be eliminated. Variables that had significant associations with the incidence of DKA were infection (OR 5.23; 95%CI 1.40 to 19.52; P=0.014) and DKA at the initial diagnosis of type 1 DM (OR 5.137; 95%CI 1.47 to 19.68; P=0.011).

Table 3. Bivariate analysis of potential predictors of DKA

Predictors	DKA (n=37)	No DKA (n=20)	OR	95% CI	P value
Sex, n					
Female	28	12	2.07	0.65 to 6.67	0.22
Male	9	8			
DKA at diagnosis of type 1 DM, n					
Yes	28	6	7.26	2.15 to 24.49	0.01
No	9	14			
Peripubertal age, n					
Yes	4	4	2.06	0.46 to 9.33	0.43
No	33	16			
Infection, n					
Yes	26	5	7.09	2.07 to 24.34	0.001
No	11	15			
Metabolic control, n					
Poor	35	16	2.83	0.57 to 14.18	0.23
Moderate + well	2	4			
Psychological problem, n					
Present	14	4	2.17	0.60 to 7.85	0.09
Absent	16	15			
Insulin therapy non-compliance, n					
Yes	11	2	3.81	0.75 to 19.28	0.09
No	26	18			
Socio-economic level, n					
Low	12	4	1.92	0.53 to 7.00	0.31
Moderate + high	25	16			
Parental education status, n					
Low	17	7	1.51	0.51 to 4.86	0.42
Moderate + high	20	13			

Table 4. Multivariate logistic regression analysis

DKA predictors	OR	95% CI	P value
Infection	6.27	1.46 to 26.66	0.01
Psychological problem	1.72	0.31 to 9.43	0.53
Poor metabolic control	2.83	0.42 to 19.06	0.29
Insulin therapy non-compliance	3.98	0.47 to 33.49	0.20
DKA at diagnosis of type 1 DM	4.77	1.19 to 19.14	0.03
Female gender	0.51	0.11 to 2.26	0.37
DKA at the initial diagnosis of type 1 DM*	5.37	1.47 to 19.68	0.011
Infection*	5.23	1.40 to 19.52	0.014

*final result

Discussion

Ketoacidosis is an acute complication of DM, especially type 1 DM, indicating a severe insulin deficiency. In our study, 37 patients (65%) with type 1 DM had DKA, similar to previous studies in other countries.[1] The incidence of DKA in type 1 DM ranges from 13 to 80%, depending on the country and geographic conditions, although this environmental effect is still unclear. The incidence rate of DKA in developing countries is higher than in developed countries.[4] Himawan *et al.*[5] in Jakarta reported that the incidence of DKA in type 1 DM patients was 76.9%, of which 56.7% had only 1 episode of DKA,

13.3% had 2 episodes of DKA, and 30% had 3 or more episodes of DKA. However, Gillani *et al.*[7] in Malaysia reported a DKA incidence of 44.5%,10 while Rewers et al. in Colorado, USA, reported a DKA incidence of 18.6% in type 1 DM patients. The occurence rate in our study was high because Indonesia is a developing country, with lower level of education, socio-economic conditions, and health care facilities than in developed countries.

The symptoms of DKA in our subjects were nausea and vomiting (89.2%), abdominal pain (73%), Kussmaul breathing (86.5%), shock (53.3%), and decreased consciousness (59.5%), similar to previous studies.[11,12] It is important to identify symptoms of DKA to recognize it as early as possible, so patients can be given appropriate treatment immediately to prevent worsening of the condition.[12]

In this study, a DKA diagnosis was established based on blood glucose > 200mg/dL, pH < 7.3, and/or HCO_3 <15 mEq/L and ketonuria.[9] We found that 54.1% of patients had severe DKA, a higher percentage than Hadi *et al.*[13] in Iraq, where 38.23% of patients had severe DKA. The high percentage of severe DKA in Dr. Sardjito General Hospital was probably because it is a central referral hospital, so referral patients tend to have severe cases.

In our study, 5 patients died (8.8%). This mortality rate was higher than in developed countries (0.15%-0.31%), but was relatively similar to that of developing countries (3.4%-13.4%).[1,2] Naveed *et al.*[6] reported that DM mortality caused by DKA in Pakistan was 7.5%. The economic and social burden caused by DKA in our study was considerable. The average length of hospitalization per episode of DKA was 8.17 (SD 6.21) days. Around 27% of DKA patients underwent PICU treatment. Children with severe DKA and children at risk of cerebral edema should be admitted to the intensive care unit.[1]

We found two significant predictive factors for the occurence of DKA in type 1 DM patients, infection and experiencing DKA at the time of initial diagnosis of type 1 DM. The incidence of infection before the occurrence of DKA was 54.4% in our subjects, which was slightly higher than in previous studies in Pakistan (26.19%) and in Iraq (45.58%).[6,13] At the time of infection, the body secretes growth hormone, glucagon, cortisol, and epinephrine, which are counter-regulatory hormones with an anti-insulin effect. Counter-regulatory hormone secretion causes an increase in the production of glucose and ketones, such as acetone and beta-hydroxybutyrate, through gluconeogenesis and glycogenolysis. The accumulation of these ketones triggers DKA.[4] In addition, chronic hyperglycemia leads to decreased immune function, increasing a person's susceptibility to infection.[14] This process occurs continuously in DM patients. If the blood sugar is continuously high, the patient is more susceptible to infection, and the infection will consequently trigger hyperglycemia and ketoacidosis.[14]

In our study, the most common infections were urinary tract infection (UTI) (38.7%) and respiratory infection (38.7%), similar to previous reports. Urinary tract infection is the most frequent infection in DM patients. About 25% of women with DM have asymptomatic UTIs and test positive for bacteria. The most common pathogen is *E. coli.* The increased incidence of UTI is thought to be due to autonomic neuropathy, resulting in decreased detrusor activity, decreased bladder sensation resulting in bladder distention, and increased residual urine and vesicoureteral reflux, leading to recurrent UTIs.[14]

For clinical purposes, portable blood ketone measurements are recommended for outpatients with a high risk of DKA. These high-risk patients can be taught to measure blood ketones following self-measurements of blood glucose. It is recommended to measure blood ketones after self-measurement of blood in the following conditions: blood glucose > 250 mg/dL, presence of DKA-initiating conditions such as infection, and presence of early DKA symptoms such as nausea, vomiting, and abdominal pain.[15]

The limitations of this study were the retrospective design and use of secondary data from medical records, especially where data were incomplete or there was possible information bias. Also, only a few psychological disorders in the medical records were actually confirmed by psychological or psychiatric examinations. Therefore, determination of psychological disorders in the data may have been biased. Some medical records also did not include data regarding the patient's psychological condition. Data on eating disorders also could not be obtained from the medical records. Eating disorders are closely related to psychological disorders and affect blood sugar levels. In addition, data on infection were obtained

from medical record data through history, physical examination, or supporting laboratory tests, but not using gold standard examinations such as culture or polymerase chain reaction (PCR). Furthermore, compliance on insulin use was also merely judged by history or medical records.

In conclusion, 65% of patients with type 1 DM had DKA and the mortality rate was 8.8%. Infection and DKA at the initial diagnosis of type 1 DM are significant predictors of DKA incidence in type 1 DM patients. According to our study, to limit likelihood of DKA, patients with active infection and early DKA symptoms, such as nausea, vomiting, and abdominal pain, measure blood or urine ketones if random blood glucose level exceeds 220 mg/dL.[14]

Conflict of Interest

None declared.

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Original Article

Rituximab in steroid resistant nephrotic syndrome

Agomoni Chaki, Farhana Rahman, Jahanara Arju, Abdullah-Al Mamun, Tahmina Jesmin, Sayed Saimul Haque, Afroza Begum, Habibur Rahman, Golam Muin Uddin, Ranjit Ranjon Roy

Abstract

Background Nephrotic syndrome (NS) is one of the most common glomerular disease in children, characterized by massive proteinuria, hypoalbuminemia, dyslipidemia and edema. Steroid-resistant nephrotic syndrome (SRNS) and steroid-dependent nephrotic syndrome (SDNS) present challenges in pharmaceutical management. Patient need several immunosuppressant for optimal control, each of which has significant side effects and difficult to get desired results. Rituximab (RTX) is a monoclonal antibody that targets B cells and has been shown to be effective for patients with SRNS and SDNS.

Objective To see efficacy of RTX in pediatric patients with SRNS.

Method This retrospective study took place in Pediatric Nephrology Department of Bangabandhu Sheikh Mujib Medical University from July 2017 to June 2019. Patients diagnosed with SRNS who were treated with RTX and followed up for 6 months were enrolled in this study. Primary endpoint was achievement of remission after rituximab infusion; secondary endpoint was relapse-free survival rate in 6 months period following rituximab infusion.

Results Total 7 patients were recruited in this study. Among them 4 were male. Clinical and lab parameters of all patients before and after RTX were compared. Complete remission achieved in 4/7 patients, partial remission in 2/7 patients and no response in 1/7 patient. Mean number of relapse in 3 patients before RTX infusion was 3.67 (SD 0.57) and after 0.33 (SD 0.00) (P=0.038).

Conclusion RTX is a biological agent that is effective and promising drug in children with SRNS. Rituximab is useful to induce and maintain remission. **[Paediatr Indones. 2019;59:175-82; doi: http://dx.doi.org/10.14238/pi59.4.2019.175-82]**.

Keywords: *nephrotic syndrome; Rituximab; steroid resistant*

Idiopathic nephrotic syndrome (INS) is one of the most common kidney disease in children and characterized by massive proteinuria, hypoalbuminemia, dyslipidemia, and edema. Idiopathic nephrotic syndrome affects 2 to 7 new children per 100,000 children per year in Western countries, with a prevalence of 15 per 100,000 under 16 years of age.[1] In Asia, the incidence is higher, 9 to 16 cases per 100,000 children per year and 10% of INS resistant to conventional oral prednisolone.[2] Disease mechanism in INS is poorly understood, but thought to include different genetic and pathologic variants,[3-5] with polymorphic podocyte injury as a unifying feature.[6-8] All described phenotypes are considered part of a pathological continuum, from minimal lesions (minimal change disease) to podocyte depletion and glomerulosclerosis (focal and segmental glomerular sclerosis).[8]

Prednisolone is the cornerstone therapy for INS, generally induce remission within 4 weeks in approximately 90% of cases. A significant portion of steroid-

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sensitive patients are likely to relapse frequently or become dependent on steroids and develop a high risk of steroid toxicity; like hypertension, growth disturbance and glucose intolerance.[9] However, 10-20% of these patients do not respond to steroids (steroid-resistant) and have a high risk of developing end-stage renal disease.[10] In cases of such intractable NS, drugs such as cyclophosphamide, levamisole, mycophenolate mofetil, cyclosporine and tacrolimus are used to reduce steroid toxicity or overcome steroid resistance. However, many of these drugs have significant side effects and are not always effective.

Rituximab (RTX) is a genetically-engineered, chimeric, monoclonal antibody directed against the CD20 antigen on the surface of B lymphocytes, inhibits B cell proliferation and differentiation. Rituximab was first introduced in clinical practice for the treatment of Hodgkin lymphoma and autoimmune diseases.[11-14] In the past 10 years, RTX has been used to treat patients with steroid-dependent or resistant NS.[10,15-17] *The 2012 Kidney Disease Improving Global Outcomes Clinical Practice Guidelines* introduced RTX as a treatment option for steroid-dependent NS.[18] The objective of this study was to see the effectiveness of RTX in steroid-resistance nephrotic syndrome.

Methods

The subjects in this study were pediatric patients with SRNS who were treated with RTX and followed up for 6 months at a tertiary care center of Pediatric Nephrology Department of Bangabandhu Sheikh Mujib Medical University from July 2017 to June 2019. Steroid-resistant nephrotic syndrome was defined as no response after 4 weeks of steroid in adequate doses (60 mg/m^2/day) plus 3 pulses intravenous methylprednisolone at 30 mg/kg/dose. Complete remission was defined as bed side urine albumin (BSUA) nil for 3 consecutive days; partial remission was defined as BSUA (+) or trace or intermittent proteinuria. Infusion reaction was defined as all adverse events occurring within 24 hours of rituximab infusion. Initial resistance was defined as absence of remission despite 4 weeks of initial steroid treatment. Late resistance was defined as an initial response but subsequent steroid resistance. Mendoza protocol consists of methylprednisolone (30 mg/kg intravenously), administered every other day for 2 weeks, weekly for 8 weeks, every other week for 8 weeks, monthly for 9 months and then every other month for 6 months, with oral prednisolone.

Before RTX administration, patients underwent following assessments: complete blood counts, biochemical parameters (albumin, serum creatinine, liver function, electrolytes, C-reactive protein, etc.), hepatitis B and C virus titer, chest X-ray. Patient got 2-4 doses of rituximab, depending on their condition, with minimum 14 days interval between doses. Rituximab (RTX) was administered at a dose of 375 mg/m^2. To minimize the risk of infusion reaction, patient received oral acetaminophen (15 mg/kg; maximum 1 gm), intravenous hydrocortisone (8 mg/kg; maximum 500 mg) and intravenous diphenhydramine (1 mg/kg; maximum 50 mg) 30 minutes prior to RTX infusion. Rituximab was diluted to 1 mg/mL with saline and administered to patients at initial dose of 5 mL/hour and increased by 5 mL every 30 min, as tolerated with monitoring of blood pressure (BP), heart rate (HR), respiratory rate (RR) and oxygen saturation (SPO_2). Maximum infusion rate was 50 mL/hour. In the event of an infusion reaction, the attending physician decreased or stopped the infusion rate according to the severity of symptom, until symptoms disappeared. The physician also administered medical intervention, such as oxygen inhalation and anti-hypertensive medications, as clinically indicated.

Patients were followed up for at least 6 months from the initial administration of rituximab. During this period, patients' complete blood count, biochemical parameters were measured and urine analysis were performed to confirm relapse and remission. A patient was considered to have relapse if proteinuria 3+ or more for 3 consecutive days. The primary endpoint was achievement of remission after rituximab infusion and secondary endpoint was relapse-free survival rate in 6 months following administration of rituximab. Number of relapses were compared before and after administration of rituximab.

Data were collected retrospectively from patients' medical records and include patients' sex, age at diagnosis, age at starting treatment, duration of disease, immunosuppressant used at start of treatment, renal histology and number of relapses. The data were analyzed with appropriate statistical test (Pearson

correlation test, Wilcoxon signed rank test or paired T-test). Statistical significance was established at P value < 0.05.

This study was approved by the Institutional Review Board (IRB), Bangabandhu Sheikh Mujib Medical University.

Results

Table 1 shows demographic characteristics of seven patients. Patients are ordered from youngest to oldest at the time of RTX treatment. Among seven patients, 4 were male. Three patients were diagnosed as SRNS in their first attack. Two patients (no. 6 and 7) did not get calcineurine inhibitor(CNI) before RTX administration due to raised serum creatinine but they got immunosuppressant other than CNI before RTX treatment. Renal biopsy was not done in 1 patient (no. 3) due to massive ascites.

Table 2 and **3** show clinical and laboratory parameters of 7 patients before and after RTX. One patient (no.3) died 3 days after RTX due to septicemia. All patients had 3+ or 4+ proteinuria before RTX. After giving RTX it was absent in 4 patients, 1+ in 1 patient, 2+ in 1 patient (P<0.001) (**Figure 1**). All

Table 1. Demographic characteristics of patients received RTX

Patient number	Age, years	Gender	Age of disease onset, years	Duration of disease before rituximab, years	Age of onset of RTX, years	Immunosuppressants before RTX	Duration of CNI, months	Number of RTX infusion
1	3	Female	2	0.5	2.5	CNI with others	1	2
2	4	Male	0.66	2.5	3	CNI with others	1	4
3	5.5	Male	4.5	1	5.5	CNI with others	1	1
4	9.5	Male	8.75	0.25	9	Others	0	2
5	10.5	Female	3	7	10	CNI with others	1.5	2
6	11.5	Female	9.5	1.5	11	Others	0	2
7	12.5	Male	9	1.5	12	Others	0	2

Table 2. Clinical and laboratory parameters before RTX

Patient number	Proteinuria	Hematuria	Hypertension	Serum creatinine, mg/dL	Albumin, g/L	Cholesterol, mg/dL	24 hrs urinary total protein, g/m^2/day
1	+++	-	+	0.2	11	614	2.8
2	+++	-	+	0.4	15	340	2.2
3	+++	+	+	1.2	14	890	2.5
4	+++	+	+	1.2	19	225	1.2
5	+++	-	+	0.5	20	254	2.0
6	+++	+	+	5	22	384	3.2
7	+++	-	+	1.4	16	549	2.2

Table 2. Clinical and laboratory parameters after RTX infusion

Patient number	Proteinuria	Hematuria	Hypertension	Serum creatinine, mg/dL	Albumin, g/L	Cholesterol, mg/dL	24 hrs urinary total protein, g/m^2/day
1	Absent	-	-	0.2	42	350	0
2	Absent	-	-	0.2	38	220	0
3							
4	+	+	+	0.6	30	210	0.5
5	Absent	-	-	0.4	36	180	0
6	++	+	+	3.8	28	340	0.8
7	Absent	-	-	0.5	40	300	0

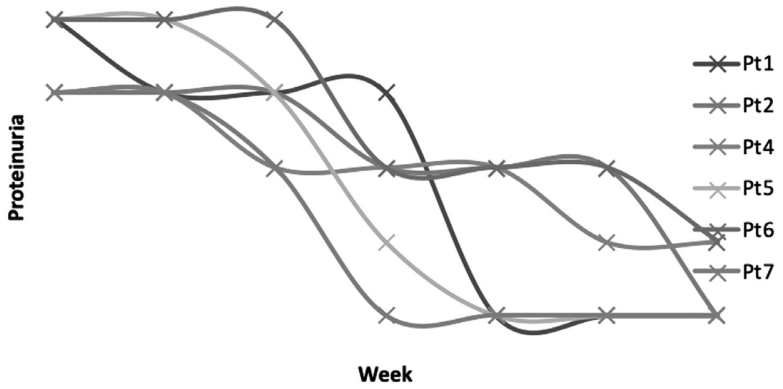

Figure 1. Status of proteinuria after RTX therapy (P ≤ 0.001) - Pearson correlation test

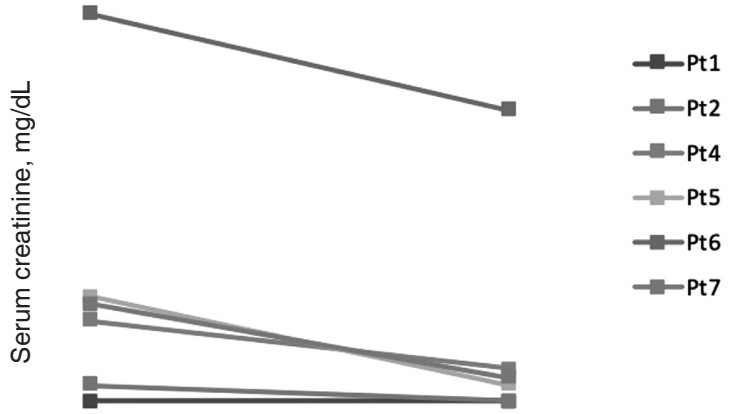

Figure 2. Pre- and post-RTX serum creatinine (P = 0.043 : Wilcoxon signed rank test)

patient had hypertension before RTX that resolved in 4 patients during follow-up period. Hematuria was present in 3 patients before RTX but it did not resolve after RTX therapy. Four patients (no. 3, 4, 6, and 7) had raised serum creatinine before RTX which became normal in 2 patients (no. 4 and 7) and decreased in 1 patient (no. 6) (P=0.043) (**Figure 2**) after RTX. Regarding renal histology, 2 patient had minimal change disease (MCD) (no. 1 and 6), 2 had mesangial proliferative glomerulonephritis (MesPGN) (no. 4 and 5), 1 had focal segmental glomerulosclerosis (FSGS) (no. 7), 1 had membranoproliferative glomerulonephritis (MPGN) (no. 2).

Figure 3 shows the primary endpoints: 4/7 patients achieved complete remission, 2/7 achieved partial remission, 1/7 showed no response (no.3) who died due to septicemia 3 days after infusion of first dose.

Table 4 shows infusion reaction (IR) during and after RTX administration. The severity of IR was categorized as grade 1-5, using the *Common Terminology Criteria for Adverse Events (CTCAE) ver. 4.0.*[19] Four patients required neither decreased nor discontinuation of RTX infusion. No patients experienced CTCAE grade 1 events. The CTCAE grade 2 events occurred in 4 patients and required non-invasive intervention such as anti-hypertensive agents and oxygen supplementation. No patients experienced grade 3-5 events and all patients completed their RTX infusion.

Table 5 shows adverse effect of RTX administration. Granulocyte count decreased in 1 patient, so granulocyte colony stimulating factor (GCSF) was given to that patient. One patient developed septicemia and died 3 days after RTX administration.

Table 4. Infusion reaction of RTX administration (n=7)

Reaction	RTX administration			
	1st dose infusion	2nd dose infusion	3rd dose infusion	4th dose infusion
Fever	0	0	0	0
Cough	0	0	0	0
Rash	0	0	0	0
Shivering	0	0	0	0
Headache	0	0	0	0
Dyspnea	2	1	0	0
Desaturation	0	1	0	0
No reaction	5	5	1*	1*

*Only 1 patient (patient no. 2) got 4 doses of RTX

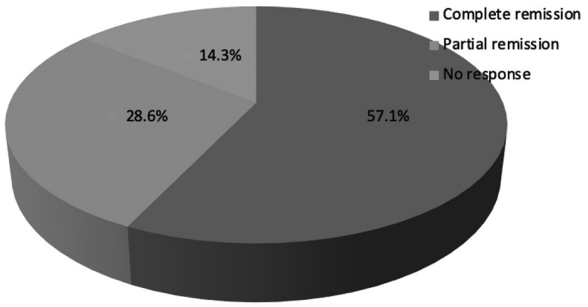

Figure 3. Effect of RTX administration

One patient developed pneumonia just after the 2nd dose of RTX infusion and clinical condition did not improve. However after 7 days that patient achieved partial remission.

Figure 4 shows secondary endpoint . Secondary endpoint was relapse-free survival rate after RTX administration. Six months after RTX administration, remission was sustained in 6 patients (patient no. 3 died 3 days after RTX therapy) either complete or partial remission with different post-RTX immunosuppressant protocol. The mean number of relapse in 3 out of 7 patient (as 3 patient was diagnosed SRNS and got RTX during their first attack, 1 patient died 3 days after RTX therapy) was 3.67 (SD 0.57) before RTX administration and 0.33 (SD 0.00) after RTX administration (P=0.038) (**Table 6**).

Table 5. Adverse effects after RTX administration

Adverse effects	Number of patients
Agranulocytosis	0
Reduced granulocyte count	1
Infection	2
Exacerbation of atopic condition	0

Table 6. Relapse-free survival rate pre-RTX and post-RTX (n=3)

	Pre-RTX	Post-RTX	P value
Mean number of relapse (SD)	3.67 (0.57)	0.33 (0.00)	0.038

Paired T-test

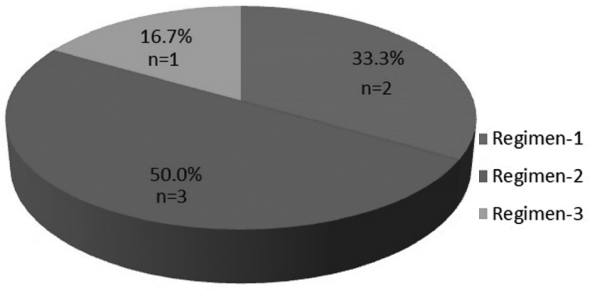

Figure 4. Patients maintaining remission over 6 months with immunosuppresant following RTX administration
Regimen-1 : Calcineurin inhibitor (CNI) + oral prednisolone
Regimen-2 : Mycophenolate mofetil (MMF) + Mendoza protocol + monthly cyclophosphamide
Regimen-3 : Calcineurin inhibitor (CNI) + Mendoza protocol + monthly cyclophosphamide

Discussion

Rituximab is considered a treatment option for SDNS and SRNS. Rituximab interacts with regulatory elements of the cytoskeleton[20] and in this way, directly modify podocyte structure. Rituximab affects regulatory elements of CD20 B cells that are implicated in innate immunity and affects Th17 cell.[21-22]

In this study, 3 patients were initialy steroid resistant and 4 patients were late steroid-resistant. All patients got 2 doses of rituximab 14 days apart (375 mg/m^2) except 1 who died 3 days after 1st infusion of rituximab. Four patient went into complete remission within 1 month of giving rituximab. This is a remarkable result in a situation where rituximab was used as a rescue therapy in patients who did not respond to immunosuppresant like prednisolone, methylprednisolone, cyclophosphamide, livamisole, MMF or CNI.

Multiple case reports support the efficacy of rituximab in inducing and/or sustaining remission of nephrotic syndrome.[23] Bagga *et al.* studied the response rate to rituximab in five pediatric patients with NS (two with MCNS and three with FSGS) who were resistant to treatment with high-dose steroids, alkylating agents and CNI. Four of these patients had a complete remission and one had partial remission. The complete remission was maintained in three patients.[16] An international, multicenter report comprising 28 patients with SDNS, 27 patients with SRNS, and 15 with post-transplant recurrence of nephrotic syndrome provided satisfactory data on efficacy and safety of rituximab.[24] Guigonis *et al*., in a multicenter report (from France), examined the efficacy of rituximab in 22 patients with SDNS or SRNS. They included a heterogeneous group of patients receiving concomitant treatment with prednisone, calcineurin inhibitors, or MMF. At a median follow-up of 9.5 months, 19 (86.3%) patients had a beneficial effect with sustained remission or reduction of proteinuria.[15] Gulati *et al*. assessed the efficacy of RTX in 33 pediatric patients with SRNS (24 with initial resistance and 9 with late resistance). Treatment consisted of 4 weekly doses of RTX (375 mg/m^2 each). Nine patients (27.2%) achieved complete remission, 7 patients (21.2%) achieved partial remission, and 17 patients (51.5%) had no response. The median time to response was 32 days (range 8 to 60) after the last dose of RTX. At the 6 months follow up, all 16 patients remained in remission (complete or partial). The remission rate was higher in patients with minimal change disease (64.7%) than in those with FSGS (31.2%; P=0.08).25 In our study, we found 1 patient with FSGS who underwent complete remission after rituximab but 1 of 2 MCD patients had partial response and 1 had complete remission. However, a previous randomized study conducted by Magnasco *et al*. showed no benefit from RTX therapy.[26] Alaifan *et al.* showed beneficial effect of RTX in SDNS in reducing relapse rate, but they concluded that RTX is less effective in SRNS.[27]

In our study, 3 patients with late SRNS went into remission following rituximab infusion and relapse significantly reduced (P=0.038) in 6-month follow-up period. Only 1 patient had 1 relapse in a year after rituximab infusion. That patient was treated successfully with re-infusion of single dose rituximab. Several studies showed rituximab is a promising agent for maintaining remission in SDNS.[28-31]

A literature review of rituximab for difficult pediatric nephrotic syndrome 32 included 9 published studies and showed RTX is relatively safe in children with nephrotic syndrome. The main adverse events were related to infusion reactions. In our study, 3 patients developed dyspnea, 1 patient developed desaturation. Reaction was mild, so infusion was continued. One of our patient developed decreased granulocyte count, so granulocyte colony-stimulating factor (GCSF) was given. But the patient was concurrently treated with IV cyclophosphamide, so it was not clear that decreased granulocyte count was an isolated effect of RTX. One patient died 3 days after rituximab infusion, as disease course worsened with severe anasarca, raised serum creatinine & urea during infusion of rituximab.

Our study has some limitation such as sample size was small and it was done in a single center. We gave rituximab only to patients with SRNS refractory to MMF, alkylating agent and CNI, as treatment with rituximab is very expensive in contrast to our economic condition. So, these sample size could be considered as an important enrollment. Moreover, study follow-up period was short, so we could not rule out delayed complication of RTX like lung fibrosis. In addition, all our patients concurrently got other immunosuppressant like prednisolone, MMF, CNI. Thus, effects of these drugs could not be ruled out.

In our small study, RTX is an effective and promising drug in children with SRNS. It is safe with mild infusion reaction. Rituximab helps to induce and maintain remission. Although there was a trend toward better response in patients with MCD, in this report, we could not identify any histological finding that predicted the response to rituximab. There is a need for randomized controlled trials with larger population to further define the role of rituximab in idiopathic childhood onset NS.

Conflict of Interest

None declared.

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Original Article

Growth of HIV-exposed infants from birth to 6 months in the prevention of mother-to-child transmission program

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Abstract

Background Human immunodeficiency virus (HIV) infection is a global health issue. Most cases of HIV infection in children are acquired through transmission from HIV-infected mothers. Maternal HIV infection affects infant growth.

Objective To evaluate the first six months of growth in HIV-exposed infants born to mothers in the prevention of mother-to-child transmission (PMTCT) program.

Methods This prospective cohort study was done in 40 HIV-exposed infants born in Sanglah General Hospital, Bali. Subjects' underwent weight and length measurements at birth and monthly for 6 months. Data analyses used were repeated ANOVA test with Bonferonni post-hoc analysis for normally distributed data and Friedman test with Wilcoxon post-hoc analysis for abnormally distributed data. Correlations between birth weight and length to weight and length at 6 months of age were analyzed with Spearman's test.

Results Subjects' mean birth weight was 2,900 (SD 546) grams and median birth length was 48 (range 36-52) cm. Subjects' body weight and length increased monthly throughout the measurement period ($P<0.001$). There was a strong negative correlation between birth weight and infant weight gain at 6 months of age ($r=-0.678$), and a moderate negative correlation between birth length and infant length gain at 6 months of age ($r=-0.564$).

Conclusion HIV-exposed infants born to mothers in the PMTCT program have a significant body weight and body length growth in the first 6 months of life, and followed general WHO weight and length curves for age. **[Paediatr Indones. 2019;59:183-7; doi: http://dx.doi.org/10.14238/pi59.4.2019.183-7]**.

Keywords: *HIV-exposed infant; body weight; body length*

Human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) are worldwide health problems, due to high transmission of infection, morbidity, and mortality. *The World Health Organization* (WHO) reported that 3.3 million children have HIV infection,[1] with most infections occurring perinatally.[2] HIV transmission from mother to child in Indonesia was reported to be 3.76%, and continues to increase every year.[3] The main pathways of HIV infection transmission to children are intrauterine, intrapartum, and post-natal (breastfeeding).[4] Maternal HIV infection affects infant growth. Several studies have shown that HIV-exposed infants had higher risk of morbidity and mortality than HIV-unexposed infants, but another US study showed similar growth in HIV-exposed uninfected infants and HIV-unexposed infants. The PMTCT program has been effective in decreasing HIV transmission risk 1-2%.[5]

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Methods

A prospective cohort study on HIV-exposed infants was conducted to evaluate growth within the first 6 months of life. This study was conducted at Sanglah Hospital, Denpasar, Bali, from April 2017 to August 2018. The inclusion criteria were HIV-exposed infants, born to HIV-infected mothers in the PMTCT program. Exclusion criteria were infants with major congenital abnormalities or whose parents did not agree to participate. Drop out criteria were skipping the measurement schedule two or more times, or being diagnosed with presumptive HIV during the study period. Data analyses in this study consisted of descriptive statistical and bivariate analyses. Comparative analyses were done using repeated measurement ANOVA test with Bonferonni post-hoc analysis for normal data distribution, and Friedman test with Wilcoxon post-hoc analysis for abnormal data distribution. Correlative analysis was done by Spearman's test. Infant growth rates were expressed using the infant weight gain percentage (IWG%), and defined as [(infant weight − birth weight)/ birth weight x 100], as well as the infant length gain percentage (ILG%) defined as [(infant length – birth length)/birth length x 100]. All data were analyzed using *SPSS version 23 software*. Results with P values <0.05 were considered to be statistically significant. This study was approved by the Research Ethics Committee of the Universitas Udayana Medical School/Sanglah Hospital.

Results

From 50 patients, 40 were included in the analysis, in accordance with the required minimum sample size calculation. The basic characteristics of subjects and their mothers are shown in **Table 1**. Subjects were mostly boys (60%), born full-term (95%), without asphyxia (92.5%), and with birth weight ≥ 2,500 grams (80%).

Comparative analysis for infant weight from birth to 6 months with repeated measurement was done with ANOVA test, followed by Bonferoni post-hoc analysis (**Table 2**). Mean weight significantly increased in at least 2 periods of time throughout the measurement period (P<0.001). Body weight growth in HIV-exposed infants in the PMTCT program followed the growth curve for weight-to-age on the WHO growth chart.

Table 1. Characteristics of subjects and mothers

Characteristics	(N=40)
Subjects	
Males, n (%)	24 (60)
Full-term, n (%)	38 (95)
Vigorous baby, n (%)	37 (92.5)
Mean birth weight (SD), grams	2900 (80)
Median birth length (range), cm	48 (36-52)
Mothers	
Mean age (SD), years	28 (4)
Multiparous, n (%)	32 (80)
Mean CD4 level (SD), celLs/μL	423 (192)

Table 2. Mean infant body weight by age

Age, months	Mean body weight (SD), g	P value*
0	2,900 (546)	<0.001
1	3,832 (826)	
2	4,717 (908)	
3	5,660 (908)	
4	6,375 (855)	
5	7,082 (945)	
6	7,655 (975)	

*repeated measurement (ANOVA) test

Comparative analysis for length from birth to 6 months was done with Friedman test, followed by Wilcoxon post-hoc analysis (**Table 3**). Median body length significantly increased in at least 2 periods of time throughout the measurement period (P<0.001). Body length growth in HIV-exposed infants in the PMTCT program followed the length-to-age growth curve of the WHO growth chart. Correlative analysis between subjects' birth weight and weight gain at 6 months of age was done with Spearman's test for

Table 3. Infant body length based on age

Age, months	Median body length (range), cm	P value*
0	48 (36-52)	<0.001
1	53 (40-59)	
2	56 (45-65)	
3	60 (51-66)	
4	63 (56-69)	
5	64.5 (60-71)	
6	67 (64-74)	

*Friedman test with Wilcoxon post-hoc analysis

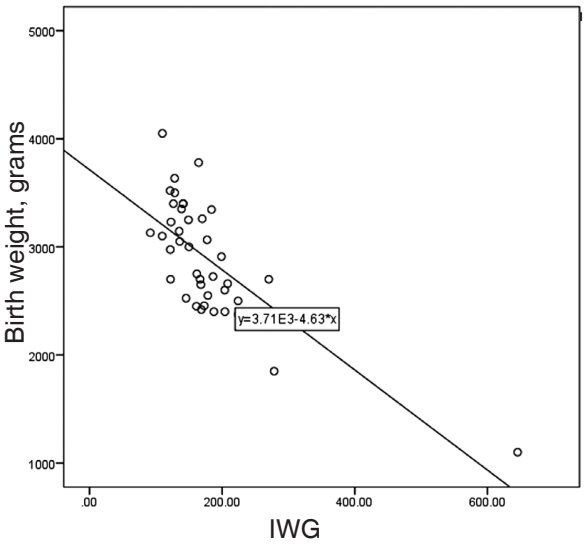

Figure 1. Correlation between birth weight and weight gain at 6 months

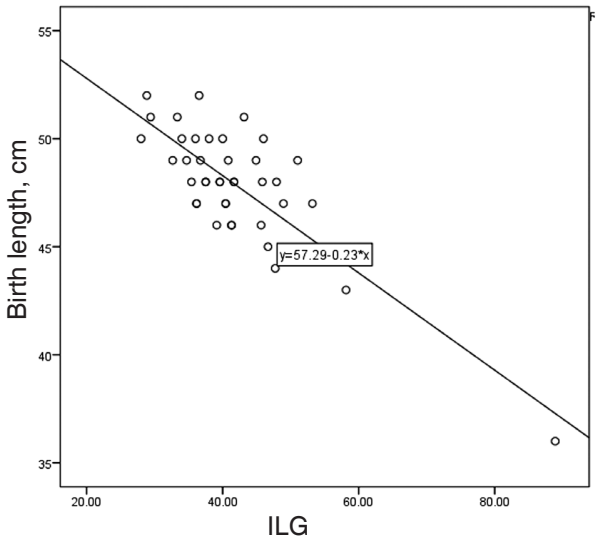

Figure 2. Correlation between birth length and length gain at 6 months

abnormal data distribution. The median IWG% was 163 (range 92-645%) with r=–0.678, which indicates a negative and strong correlation; lower birth weight was correlated to higher weight growth at 6 months. Spearman's test revealed a median ILG% of 40% (28-89%), with r=–0.564, which indicates a negative and moderate correlation; lower birth length was correlated to the higher length growth at 6 months.

Discussion

Subjects were forty HIV-exposed infants in the PMTCT program, comprising 60% boys, 95% full-term, 92.5% without asphyxia, and 80% with birth weight ≥ 2,500 grams. Subjects' mean birth weight was 2,900 grams and mean weight-for-age *z*-score (WAZ) was -0.86. Subjects' mean birth length was 48 cm and mean length-for-age *z*-score (LAZ) was -0.78. A previous study in 2016 show similar results, with median WAZ in HIV-exposed infant of -0.65 (-1.46) at birth.[9]

Long-term nutritional and neurodevelopmental outcomes are influenced by environmental factors during the first 1,000 days after conception - the period from conception to 2 years of age. HIV exposure during this period could, therefore, crucially affect birth, growth, and development. In developing countries, HIV-exposed, uninfected newborns are likely to be small-for-gestational age or have low birth weight compared to HIV-unexposed newborns.[5] Anti-retroviral exposure in HIV-infected mothers was associated with significantly lower mean LAZ and lower head circumference for age z score (HCAZ) at the age of one year, but not at birth. Siberry *et al.*[6] also found no association between antiretroviral exposure during pregnancy and lower weight, shorter length, or smaller head circumference in the newborn period.

In our study, subjects significantly increased in weight and length throughout the measurement period during the first six months of life (P<0.001). The median weight and length based on monthly age for the first six months followed the WHO growth chart curve, but was still below the curve. A previous study showed no difference in growth between HIV-exposed, uninfected infants and HIV-unexposed infants.[7] However, Evans *et al.*[5] showed that HIV-exposed infants tended to be small-for-gestational age, or have lower birth weight than HIV-unexposed infants. The PMTCT program is a strategy to reduce HIV infection transmission from mother to baby. The program includes antiretroviral (ARV) therapy for the mother, Caesarean section delivery, anti-retroviral and cotrimoxazole prophylaxis for the infant, and formula milk for infant nutrition.

Current national and international reference growth charts are based on cross-sectional data. Their primary limitation is that they do not monitor

growth as such, but identify infants whose weight and length centiles are considered low and/or falling. A better approach for monitoring infant growth would be based on longitudinal data - that is, comparisons of current growth measures with previous growth measures to assess whether or not infants are growing at a faster or slower rate.[8] Infant weight gain percentage (IWG%) could be used as an alternative to assess infant weight gain, as can infant length gain percentage (ILG%) be used to assess length gain. Birth weight and length are an important determinants for infant growth. By expressing the weight gain as a percentage of birth weight, IWG% has an advantage over a simple measure of weight gain, as it represents the extent of an infant's weight gain relative to birth weight.[8]

Birth weight and length appear to affect later growth. Hence, we assessed growth by measuring infant weight gain relative to birth weight, and infant length gain relative to birth length. We found negative correlations between birth weight and weight growth at 6 months as well as between birth length and length growth at six months. The negative correlation means that lower birth weight and birth length was related to higher weight and length gain at 6 months old. Similarly, Borah et al. reported that lower birth weight and birth length babies had higher weight and length gain at 6 months old.[9] Another study said that very low birth weight infants showed catch-up growth during the first year, but their weight and length remained less than full-term peers. Significant catch-up growth for weight and length was observed during the first year with mean *z*-score change of 0.40 (SD 1.05) and 1.01 (SD 1.25), respectively.[10] The strong correlation in weight gain at 6 months and moderate correlation in length gain at 6 months could have been caused by slower length growth than weight growth.

Body weight is one of clinical indicators used to decide when to stop prophylaxis co-trimoxazole in HIV-exposed infants. The WHO guidelines recommend co-trimoxazole prophylaxis for HIV-exposed infants aged 4-6 weeks, to be continued until HIV infection has been excluded by an age-appropriate HIV test to establish a final diagnosis or until 6 months - 1 year of age if children's growth, development, and health status are good. In other words, good weight gain in HIV-exposed infants could be a determining factor to stop prophylaxis cotrimoxazole earlier.

The limitations of this study were not comparing growth to HIV-unexposed infants, and not analyzing the HIV infection status of the infant. Because the virology examination that can be done at 6 weeks on HIV-exposed infants is not a routine procedure, so it was not possible to test them before the study. The routine procedure to diagnose HIV in HIV-exposed infant is usually done at 18 months of age by HIV antibody test. We also did not evaluate the head circumference of subjects as a growth parameter in infants.

In conclusion, HIV-exposed infants born to mothers in the PMTCT program have a significant body weight and body length growth in the first 6 months of life.

Conflict of Interest

None declared.

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Original Article

Implementing *Yogyakarta Pediatric Cancer Registry* for 16 Years: a report of hospital-based childhood cancer registry

Sri Mulatsih, Adnina Hariningrum, Ignatius Purwanto

Abstract

Background A hospital-based cancer registry can be used as a guide to decision-making. Considering the limited cancer registry data in the population, the *Yogyakarta Pediatric Cancer Registry* (YPCR) is one of the pioneers of hospital-based pediatric cancer registries in Indonesia. The YPCR was started in 2000 in Dr. Sardjito Hospital.

Objective To describe the characteristics of childhood cancer and the outcomes by analyzing overall survival (OS) and event-free survival (EFS) based on data from Yogyakarta Pediatric Cancer Registry.

Methods Data were collected from the YPCR for the period of 2000 to 2016. Childhood cancers were classified into 12 groups based on the *3rd edition International Classification for Childhood Cancer* (ICCC). Incidence, frequency, and distribution of cases were grouped by sex, age, and patients' place of residence. Incidence was further analyzed using SPSS software. Kaplan-Meier test was used to analyze OS and EFS.

Results Within the study period, 2,441 children aged 0-18 years were diagnosed with cancer. The highest incidence was found in the 1-5-year age group. The most common diagnoses found were leukemia, myeloproliferative disorders, and myelodysplastic disease (58%); lymphoma and reticuloendothelial neoplasm (8%); retinoblastoma (6%); soft tissue and other extra-osseous sarcomas (5%); as well as neuroblastoma and other peripheral nervous cell tumors (5%). The OSs of acute lymphoblastic leukemia (ALL), high risk ALL (HR-ALL), and standard risk (SR-ALL) were 31.8%, 18.5%, and 43.9%, respectively. The EFSs of ALL, HR-ALL, and SR-ALL were 23.9%, 14.7%, and 32.4%, respectively. For solid tumors, the OS was 13.7% and EFS was 6.4%.

Conclusion The number of new cases of childhood cancer has increased in the last few years. The Yogyakarta Pediatric Cancer Registry (YPCR), which serves as a hospital-based pediatric cancer registry, has an important role to evaluate clinical and non-clinical aspects of childhood cancer. **[Paediatr Indones. 2019;59:188-94; doi: http://dx.doi.org/10.14238/pi59.4.2019.188-94]**.

Keywords: *childhood cancer registry; hospital-based cancer registry; childhood cancer incidence*

The trend in childhood cancer incidence has increased in recent years.[1] In Switzerland, there were more than 2,000 new cases of childhood cancer, with more than 80% of Swiss resident aged <15 years. Ninety-two percent of the cases were malignant. Leukemia was the most common cancer found, followed by central nervous system (CNS) malignancy, lymphoma, and neuroblastoma.[2] In Japan, there were more than 1,500 new childhood cancer cases from 2009-2011. Leukemia

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was the leading diagnosis found in the study (38.1%), followed by CNS malignancy, lymphoma, germ cell and other gonadal tumors, and neuroblastoma.[3] In the other hand, survival rates of childhood cancer patients have increased in the past few decades. In 1960, the 5-year survival rate of patients with childhood cancer was less than 30%, but the current 5-year survival of such patients is more than 80%, indicating successful therapy in recent years.[4] Such data can only be obtained from a well-maintained cancer registry, which could be either population-based or hospital-based.

Cancer registry is the key to establish cancer management strategies. It is also a reliable tool to determine the burden of disease as well as to gather the information for causative study.[5] *The International Agency for Research on Cancer* (IARC) stated that only 8% of the population in Asia has been covered by population-based cancer registry, compared to other regions, such as North America which has 90% population coverage, and Europe which has 60%. The limitations in diagnosis and therapy, lack of reliable data of the population, cultural beliefs, unstable economic and political conditions, and massive migration complicate the process of establishing a population-based registry. These limitations result in an underreported incidence rate.[6] As such, establishing a hospital-based registry may be a simpler way to obtain reliable information on childhood cancer and an important step toward population-based registry data collection. The data from a hospital-based cancer registry can be a powerful instrument for clinical epidemiology and evidence-based medicine, as well as a valuable source of information to guide decision-making. However, hospital-based patient registry cannot be used to measure real incidence and mortality in the population, since the data are only obtained from patients with access to the referral hospital.[6]

The Yogyakarta Pediatric Cancer Registry (YPCR) is based on patient data from the Pediatric Wards in Dr. Sardjito Hospital, Yogyakarta, which serves as a referral hospital for many pediatric cancer patients in Indonesia. In 2001, a twinning project between Universitas Gadjah Mada and the *Saskatchewan Cancer Agency*, Canada, was launched to create a computerized cancer registry at Dr. Sardjito Hospital. Before this project began, Indonesia did not have any pediatric cancer registry, so we had little data to measure incidence, mortality, and outcomes, or to evaluate the clinical and non-clinical aspects of pediatric cancer. The objectives of this project were to develop a hospital-based, computerized pediatric cancer registry, and to compare demographics of childhood cancers between the hospital-based YPCR and the population-based *Saskatchewan Cancer Registry.*[7]

This study marks 16 years of YPCR data collection. The aim of this study was to describe the characteristics of childhood cancer in Dr. Sardjito Hospital, and to investigate treatment outcomes of childhood cancer by analyzing overall survival (OS) and event-free survival (EFS). We hope that this study will bring more improvements and innovation in the YPCR and a nationwide, population-based childhood cancer registry in the future.

Methods

Data were collected during 2000-2016 from the YPCR. Trained registrars update the data from patients in Pediatric Wards at Dr. Sardjito Hospital into YPCR. Data such as patients' characteristics, demographic information, diagnoses, cancer treatments, follow-up status, and the use of traditional healing practices were recorded in the registry. Quality control was performed to ensure the completeness of records, consistent data, and no duplications.

Childhood cancer was defined as patients aged 0-18 years who were diagnosed with cancer according to the *International Classification of Disease Oncology* (ICD-O), 3rd edition.[8] Diagnoses were further classified based on the *International Classification of Childhood Cancer* (ICCC), 3rd edition[9] into 12 main groups: I) Leukemias, myeloproliferative diseases (MPD), and myelodysplastic diseases (MDD); II) Lymphomas and reticuloendothelial neoplasms; III) CNS and miscellaneous intra-cranial and intra-spinal neoplasms; IV) Neuroblastoma and other peripheral nervous cell tumors; V) Retinoblastoma; VI) Renal tumors; VII) Hepatic tumors; VIII) Malignant bone tumors; IX) Soft tissue and other extraosseous sarcomas; X) Germ cell tumors, trophoblastic tumors, and neoplasms of gonads; XI) Other malignant epithelial neoplasms and malignant melanomas; and XII) Other and unspecified malignant neoplasms. The diagnosis was made through clinical examination, pathology report, as well as imaging examination and other supporting laboratory test.

New cases, frequency, and distribution of cases were grouped by sex and age (<1 year, 1-5 years, 6-9 years, 10-14 years, and 15-18 years). These groupings were made in order to assess cancer trends based on sex and age. The cases were also grouped by patients' residence, in order to track the regions of referral. The frequency and distribution of the cases was analyzed using *SPSS* software, while 16-year and 6 year OS and EFS were analyzed using Kaplan-Meier test. Survival analyses of ALL and solid tumors were calculated at 16 and 6 years of observation.

Results

Total of new cases of childhood cancer recorded in the YPCR increased from 2000-2016 (**Figure 1**). During that period, there were 2,441 children aged 0-18 years

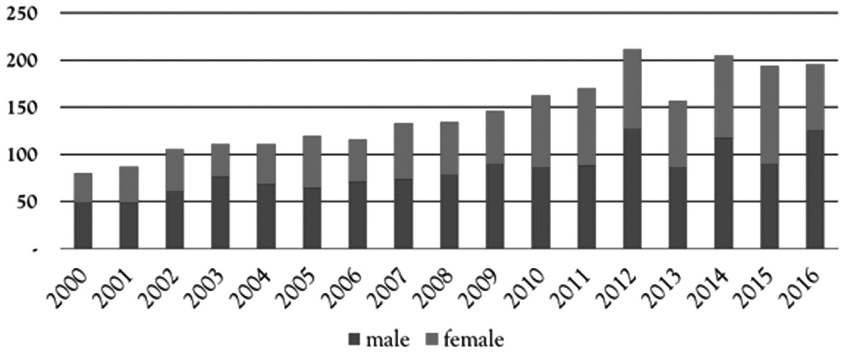

Figure 1. Childhood cancer new cases in Dr. Sardjito Hospital per year

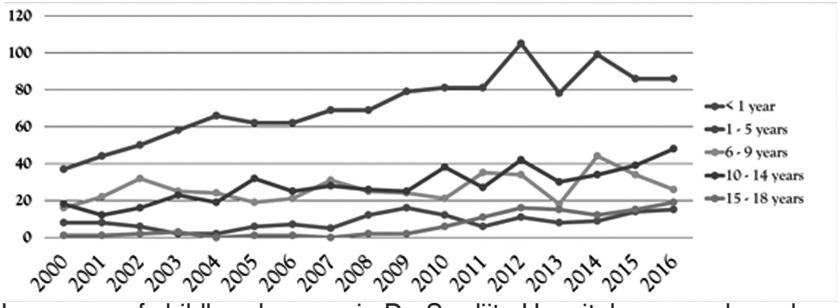

Figure 2. New cases of childhood cancer in Dr. Sardjito Hospital per year based on age group

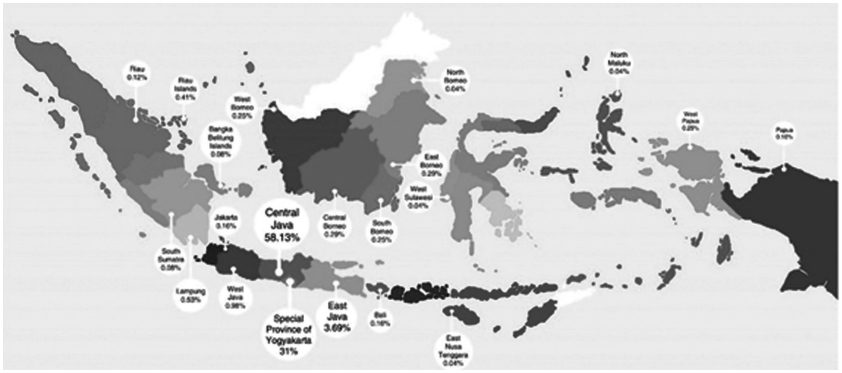

Figure 3. Referral origin of childhood cancer patients in Dr. Sardjito Hospital

were diagnosed with cancer. The largest number of new cases were found in the 1-5-years age group (49,7%), followed by the 10-14-years age group (19,7%), then the 6-9-years age group (18,5%) (**Figure 2**). Overall, the number of new cases in every age group increased in the past few years, especially in the 15-18-year group, which escalated almost 20 times in 16 years of study. The most common diagnoses were leukemia, MPD and MDD (58%); followed by lymphoma and reticuloendothelial neoplasm (8%); retinoblastoma (6%); soft tissue and other extra-osseous sarcomas (5%); and neuroblastoma and other peripheral nervous cell tumors (5%) (**Table 1**). The total number of male patients was 1,398 and female patients was 1,043 (**Table 1**). Each year, males predominated the childhood cancer population (**Figure 1**). The male to female ratio was 1.34 to 1. Most patients referred to Dr. Sardjito Hospital came from Central Java (58.13%), Yogyakarta Special Province (31%), and East Java (3.69%), however, many also came from other regions throughout Indonesia (**Figure 3**).

Trends in diagnoses varied by age group (**Figure 4**). The most common childhood cancers found in < 5 years age group were in the leukemia, MPD, and MDD category, particularly ALL, followed by nephroblastoma, hepatic tumor, and neuroblastoma and other peripheral nervous cell tumors. In 5-9 years age group, the most common childhood cancers were leukemia, MPD and MDD, mostly ALL; then lymphoma and reticuloendothelial neoplasms, out of whom 67% were non-Hodgkins lymphoma (NHL), followed by soft tissue and other extraosseous sarcomas, in which all cases were diagnosed with rhabdomyosarcoma, and neuroblastoma and other peripheral nervous cell tumors. In 10-14 years age group, the most common childhood cancers were leukemia, MPD and MDD, of which ALL accounted

Table 1. Childhood cancer incidence in Dr. Sardjito Hospital

Characteristics	N=2,441
Sex	
Male	1,398 (57.3)
Female	1,043 (42.7)
Age group	
< 1 year	147 (6.02)
1-5 years	1,212 (49.7)
6 -9 years	451 (18.5)
10 - 14 years	482 (19.7)
15 - 18 years	107 (4.4)
No data	42 (1.7)
Diagnosis	
I. Leukemias, myeloproliferative diseases, and myelodysplastic diseases	1,427 (58.5)
II. Lymphomas and reticuloendothelial neoplasms	190 (7.8)
III. CNS and miscellaneous intracranial and intra-spinal neoplasms	81 (3.3)
IV. Neuroblastoma and other peripheral nervous cell tumors	112 (4.6)
V. Retinoblastoma	147 (6.0)
VI. Renal tumors	103 (4.2)
VII. Hepatic tumors	41 (1.7)
VIII. Malignant bone tumors	49 (2.0)
IX. Soft tissue and other extraosseous sarcomas	133 (5.4)
X. Germ cell tumors, trophoblastic tumors, and neoplasms of gonads	105 (4,3)
XI. Other malignant epithelial neoplasms and malignant melanomas	41 (1.7)
XII. Other and unspecified malignant/ neoplasms	12 (0.5)

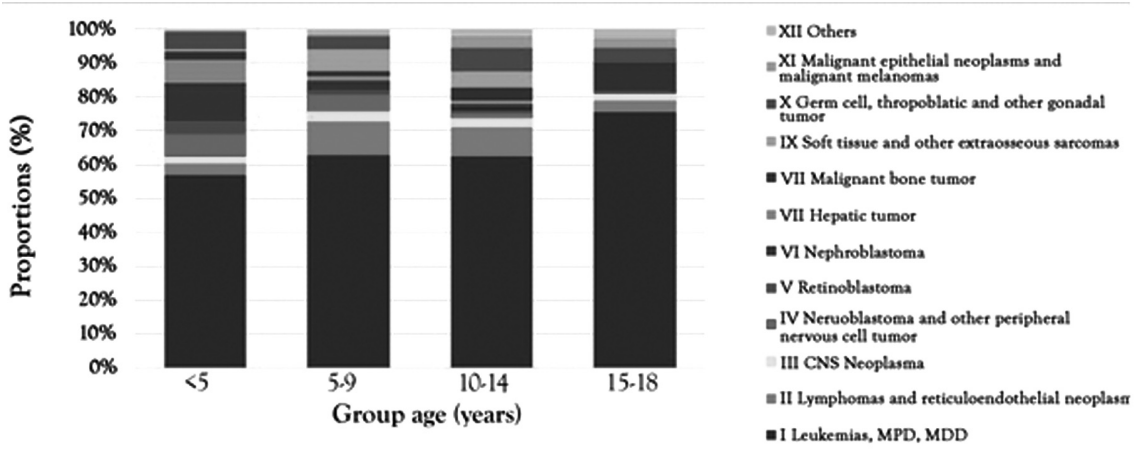

Figure 4. Proportion of childhood cancer cases based on group age

for more than half of the cases; followed by lymphoma and reticuloendothelial neoplasms, with NHL as the most common diagnosis; germ cell, trophoblastic and other gonadal tumors, with most cases diagnosed to have teratoma; and lastly, soft tissue and other extraosseous sarcomas, mostly diagnosed with rhabdomyosarcoma. The most common childhood cancers found in 15-18 years age group were leukemia, MPD, and MDD, with ALL as the most common diagnosis; malignant bone tumors all of whom had osteosarcoma; germ cell tumors, trophoblastic and other gonadal tumors, with 2 cases of teratoma and 1 case of ovarian carcinoma; and lastly lymphoma and reticuloendothelial neoplasms, in which all the cases were NHL. In total, leukemia, more specifically ALL, had the highest incidence of all childhood cancer, in every age group.

In the past 16 years, childhood cancer incidence trends increased year by year. The highest incidence was in 2012 and the lowest was in 2013. The total of the leukemia, MPD and MDD group's new cases had the highest increment, with a 3 times higher new cases in 2016 compared to that in 2000, when the YPCR started. New cases of leukemia were mostly found in the <5 years age group, which accounted for half of the total incidence of leukemia, then decreased with subsequent increase in age groups.

Survival rate analyses of ALL and solid tumor patients who received chemotherapy from 2000-2016 were performed at 16 and 6 years of observation. The OS rate of ALL was 31.8% (**Figure 5**), with OS rate of high risk (HR) ALL of 18.5% and OS rate of standard risk (SR) ALL of 43.9%. Furthermore, EFS of ALL during the study period was 23.9%. The EFS of HR ALL was 14.7% and the EFS of SR ALL was 32.4% (**Figure 5**). The OS rate of solid tumor was 13.7% (**Figure 5**) and the EFS was 6.4% (**Figure 6**).

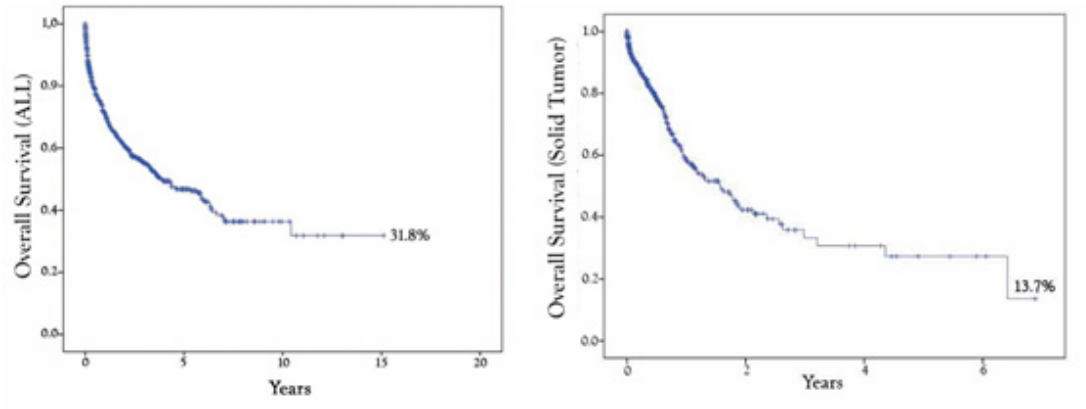

Figure 5. Overall survival of ALL and solid tumor

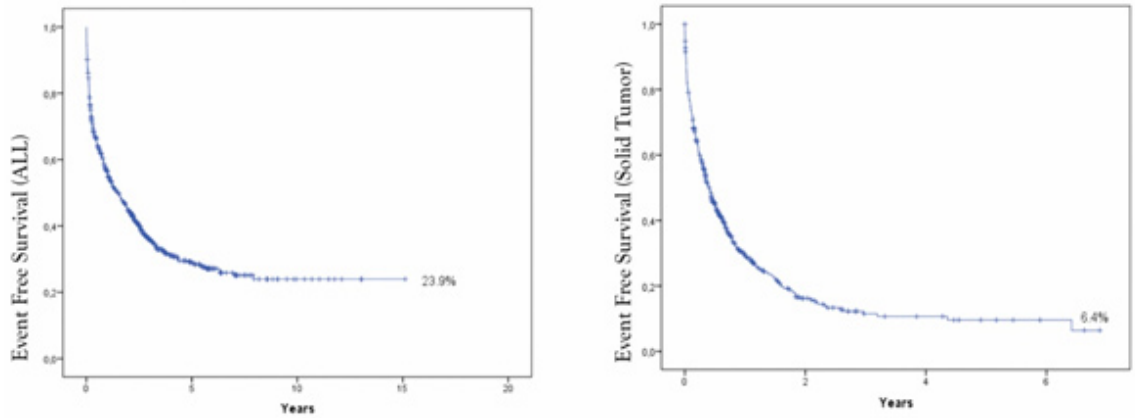

Figure 6. Event free survival of ALL and solid tumor

Discussion

Epidemiologic studies of childhood cancer are useful for revealing periods of tumor initiation by analyzing age distributions of cancer, which then provide deeper understanding about the etiology of cancer. For example, genetic syndromes and higher birth weight are well-established risk factors, but they only account for a small proportion of cases.[10,11] Moreover, survival data are also important to develop surveillance programs for cancer survivors and to determine survival trends which can be used to evaluate progress related to treatment. However, obtaining data for epidemiologic studies of childhood cancer have been difficult to achieve, particularly in Asia, due to the lack of complete, comprehensive, nationwide epidemiological reports on childhood cancer.[12]

The Yogyakarta Pediatric Cancer Registry (YPCR) was initiated in 2000 as a pioneer of computerized, hospital-based childhood cancer registry in Indonesia. It was expected to close the gap of childhood cancer epidemiological information before a nationwide, population-based childhood cancer registry could be developed. The registry has been running for 16 years to date, and has recorded data on 2,441 childhood cancer cases, including 1,398 male and 1,043 female patients. The number of childhood cancer new cases has increased yearly, for both males and females. Male patients predominated over females almost every year, with an overall 1.34:1 ratio. This finding was similar to male to female ratios in previous studies, such as in Canada which had 1.12:1, and in Australia which had 1.14:1.[13,14] In Australia, the highest incidence was found in the 0-4-year age group, while in Indonesia it was found in the 1-5-year age group.[13]

The increasing trends in childhood cancer incidence in Indonesia may be due to changes in diagnostic, coding, or registration practices,[15,16] which may have been propagated by Indonesia's recently-launched universal health coverage in 2014. National health insurance allows all residents in Indonesia to have free access to health services. Those with financial problems have access to their nearest primary health care facility, as well as to tertiary health care, if needed. The health insurance system also enables children with signs and symptoms of cancer to be referred to a tertiary hospital such as Dr. Sardjito Hospital where the YPCR is located, for further investigation and free treatment. The improvement in economic and educational status of the population may also be other factors encouraging more people to seek medical help.

We found that the leukemia, MPD, and MDD category was the most common diagnosis in childhood cancer, accounting for 58.5% of all the cases. In other countries, the leukemia percentages were 35% in Shanghai, China and Chennai, India, 33% in Germany, 30% in Ireland and France, and 27% of pediatric cancers in the United States.[17,18] Our findings were relatively similar, hence, we can conclude that leukemia is the most common cancer in childhood.

The strength of this study was the fact that YPCR is the pioneer of computerized, hospital-based childhood cancer registry in Indonesia, as Indonesia does not have a nationwide, population-based, childhood cancer registry. The weakness of this study was that the data were from one hospital, thus, not representive of epidemiological data for the region or nation. Since not everyone has access to the Dr Sardjito hospital, it was not possible to calculate childhood cancer incidence in the population. Even when health services are free, some people cannot afford transportation fees or other expenses needed during hospitalization.

In conclusion, the number of new cases of childhood cancer has increased in the last few years. A cancer registry is necessary to conduct epidemiological studies and evaluate the clinical and non-clinical aspects of childhood cancer, especially since childhood cancer new cases have increased in the last few years.

Conflict of Interest

None declared.

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Original Article

The CDC PNU-1 criteria for diagnosis of ventilator-associated pneumonia

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Abstract

Background Ventilator-associated pneumonia (VAP) is one of the most common nosocomial infections in the pediatric intensive care unit (PICU), with mortality rates of up to 50%. Post-mortem pulmonary examination is considered to be the gold standard for diagnosis of VAP, but is impossible for routine application. The sensitivity and specificity of *Clinical Pulmonary Infection Score* (CPIS) are considered to be similar to the those of the gold standard, but the *Centers for Disease Control and Prevention* PNU-1 (CDC PNU-1) is simpler and not invasive, compared to the CPIS.

Objective To evaluate the level of agreement between CDC PNU-1 and CPIS criteria in diagnosing VAP.

Methods This cross-sectional study was conducted in the PICU at Dr. Soetomo Hospital, Surabaya from June to October 2018. Subjects were children aged 1 month–18 years who had been intubated for more than 48 hours. The VAP diagnoses were made by CDC PNU-1 and CPIS criteria. The level of agreement between the two methods was evaluated by Cohen's Kappa test using SPSS Statistics Base 21.0 software.

Results Thirty-six subjects were evaluated using CDC PNU-1 and CPIS criteria. Subjects' mean age was 3.5 (SD 4.7) years. Amongst 19 patients with VAP, 14 were diagnosed by CPIS criteria and 17 were diagnosed by CDC PNU-1 criteria. The level of agreement between the CDC PNU-1 and CPIS criteria was good (Kappa 0.61; 95%CI 0.31 to 0.83). The CDC PNU-1 had sensitivity 0.85, specificity 0.77, positive predictive value (PPV) 0.70, and negative predictive value (NPV) 0.89.

Conclusion The CDC PNU-1 criteria has a good level of agreement with CPIS criteria in diagnosing VAP. **[Paediatr Indones. 2019;59:195-201; doi: http://dx.doi.org/10.14238/pi59.4.2019.195-201]**.

Keywords: *ventilator-associated pneumonia; VAP; criteria pulmonary infection score; CPIS; CDC PNU-1*

Ventilator-associated pneumonia (VAP) is one of the most common nosocomial infections in the PICU. The definition of VAP is pneumonia that develops in the patient who has been intubated and received mechanical ventilation for 48 hours or more.[1,2] Mechanical ventilation may increase the risk of hospital-acquired pneumonia (HAP) in the PICU by 6-21 times, with mortality rate of 33-50%. Variations may be associated with the patients' underlying diseases. A surveillance study by the *International Nosocomial Infection Control Consortium* (INICC) in several countries used clinical, radiological, and microbiological criteria and concluded that more VAP cases occurred in low-middle-income countries, such as India (36.2%), compared to upper-middle-income countries, such as Italy (6.6%).[3]

Post-mortem pulmonary histology and microbiological examination performed immediately after death is the gold standard in establishing a diagnosis of

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VAP. However, this examination is impossible to apply to the management of VAP patients. Several studies have been conducted to determine the best method for diagnosing VAP, the most common of which is the *Clinical Pulmonary Infection Score* (CPIS), which uses clinical, radiological, and microbiological criteria. Although the CPIS criteria require rather invasive methods, CPIS has a good diagnosis value (sensitivity 72% and specificity 85%).[4] A more recently-developed diagnostic tool that is simpler and non-invasive compared to CPIS is the *Centers for Disease Control and Prevention* (CDC) PNU-1. It has never been used in Indonesia. This CDC criteria is non-invasive and only uses clinical criteria, without microbiological examination. Since 2009, these criteria have been used in various institutions with specific advantages for certain age groups.[5]

Guidelines for diagnosis and management of VAP in pediatric patients is very limited.[6,7] The choice of simple, inexpensive, non-invasive, and fast diagnostic tools is needed, especially in low-income countries.

This study aimed to evaluate the level of agreement between CDC PNU-1 and CPIS criteria in diagnosing VAP.

Methods

A cross-sectional study was conducted in the PICU of Dr. Soetomo General Hospital, Surabaya, from June to October 2018. Subjects were children aged 1 month–18 years who were intubated and had mechanical ventilation for 48 hours or longer. The observations for 48 hours were carried out by assessing each item in each criteria (**Table 1** and **2**). Diagnoses of VAP were made by both CDC PNU-1 and CPIS criteria (VAP or no VAP). Ventilator-associated pneumonia was considered to be established for CPIS score >6. Exclusion criteria were the presence of pneumonia prior to ventilation, immunocompromised status (absolute neutrophil count or total white blood cell count < 500/mm^3), leukemia, lymphoma, HIV with CD4 < 200 cells/mm^3, or those who had a history of solid organ or hematopoietic stem cell transplant, splenectomy, cytotoxic chemotherapy, or steroid use (excluding inhaled steroids) daily for > 2 weeks on the date of VAP established.

This study was approved by the Ethics Committee at Dr. Soetomo Hospital. Characteristic data collected included age, gender, length of PICU stay, duration of mechanical ventilation, frequency of intubation, difficulty intubating, PRISM 3 score, and main disease. Subjects were evaluated for VAP by both criteria (**Tables 1** and **2**).

The level of agreement was analyzed by Cohen's Kappa statistic (κ), which is a robust tool for measuring observational correlation, taking into account the variation due to chance. Standard error for κ was calculated using the original equation proposed by Cohen.[10] Kappa values (κ) of <0.20 show poor agreement, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.80 good, and 0.81-1.00 very good agreement. Statistical analysis was performed using *IBM SPSS statistics version 20* software. Chi-square test was used to compare

Table 1. Clinical pulmonary infection score (CPIS) criteria[8]

CPIS	Skor
Temperature (°C)	
> or equal to 36.5 and < or equal to 38.4	0
> or equal to 38.5 and < or equal to 38.9	1
> or equal to 39 and < or equal to 36	2
Blood leukocytes, mm3	
> or equal to 4,000 and < or equal to 11,000	0
< 4,000 or > 11,000	1
+ band forms > equal to 50%	Add 1 point
Tracheal secretions	
Absence of tracheal secretions	0
Presence of non-purulent tracheal secretions	1
Presence of purulent tracheal secretions	2
Oxygenation: PaO2/FIO2, mmHg	
> 240 or ARDS	0
< or equal to 240 and no ARDS (ARDS defined as PaO2/FIO2 , < or equal to 200, pulmonary arterial wedge pressure < or equal to 18 mmHg and acute bilateral infiltrates)	2
Pulmonary radiography	
No infiltrate	0
Diffuse (or patchy) infiltrate	1
Localized infiltrate	2
Progression of pulmonary infiltrate	
No radiographic progression	0
Radiographic progression (after CHF and ARDS excluded)	2
Culture of tracheal aspirate	
Pathogenic bacteria cultured in rare or light quantity or growth	0
Pathogenic bacteria cultured in moderate or heavy quantity	1
Same pathogenic bacteria seen on Gram stain	Add 1 point

Table 2. CDC PNU-1 criteria[9]

Signs/symptoms/laboratory
For ANY PATIENT, at least one of the following: • Fever (>38.0°C or >100.4°F) • Leukopenia (≤4000 WBC/mm^3) or leukocytosis (>12,000 WBC/mm^3) • For adults >70 years old, altered mental status with no other recognized cause And at least two of the following: • New onset of purulent sputum or change in character of sputum, or increased respiratory secretions, or increased suctioning requirements • New onset or worsening cough, or dyspnea, or tachypnea • Rales or bronchial breath sounds • Worsening gas exchange (for example: O_2 desaturations (for example: PaO_2/FiO_2 < 240), increased oxygen requirement, or increased ventilator demand) ALTERNATE CRITERIA, for infants < 1 years old : Worsening gas exchange (for example : desaturation (for example oximetry < 94%), increased oxygen requirements, or increased ventilator demand) And at least three of the following : • Temperature instability • Leukopenia (< 4000 WBC/mm^3) or leukocytosis (> 15,000 WBC/mm^3) and left shift (>10% band forms) • New onset of purulent sputum or change in character of sputum, or increased respiratory secretions or increased suctioning requirements • Apnea, tachypnea, nasal flaring with retraction of chest wall or nasal flaring with grunting • Wheezing, rales, or rhonchi • Cough • Bradycardia (<100 beats/min) or tachycardia (170 beats/min) ALTERNATE CRITERIA, for child >1 year old or ≤12 years old, at least three of the following: • Fever (>38. 0°C or >100. 4°F) or hypothermia (< 36. 0°C or <96.8°F) • Leukopenia (≤4000 WBC/mm^3) or leukocytosis (≥15,000 WBC/mm^3) • New onset of purulent sputum or change in character of sputum, or increased respiratory secretions, or increased suctioning requirements • New onset or worsening cough, or dyspnea, apnea, or tachypnea • Rales or bronchial breath sounds • Worsening gas exchange (for example: O_2 desaturations [for example pulse oximetry < 94%), increased oxygen requirements, or increased ventilator demand)
Imaging test evidence
Two or more serial chest imaging test results with at least one of the following: New and persistent or Progressive and persistent • Infiltrate • Consolidation • Cavitation • Pneumatoceles, in infants ≤1 year old Note: In patients without underlying pulmonary or cardiac disease (for example: respiratory distress syndrome, bronchopulmonary dysplasia, pulmonary edema, or chronic obstructive pulmonary disease), one definitive imaging test result is acceptable

proportion and Mann-Whitney U test was used to compare quantitative variables.

Results

Of 50 mechanically-ventilated PICU patients during the study period, 36 met the inclusion criteria. Fourteen patients were excluded, 10 because of pneumonia prior to ventilation, 2 due to their immunocompromised status, and 2 because they died before a VAP diagnosis was established. The study flow chart is shown in **Figure 1**. Characteristics of subjects are listed in **Table 3**.

Nineteen patient were diagnosed as VAP with both criteria. Fourteen were diagnosed with VAP by CPIS and 17 patients by the CDC PNU-1 criteria. The most common bacterial cause of VAP was Acinetobacter baumanii, which was found in 6/36 children.

The CDC PNU-1 criteria showed a good level of agreement with CPIS (Cohen's κ=0.61; 95%CI 0.31 to 0.83; P<0.001). The CDC PNU-1 criteria had a sensitivity of 0.86 and a specificity of 0.77.

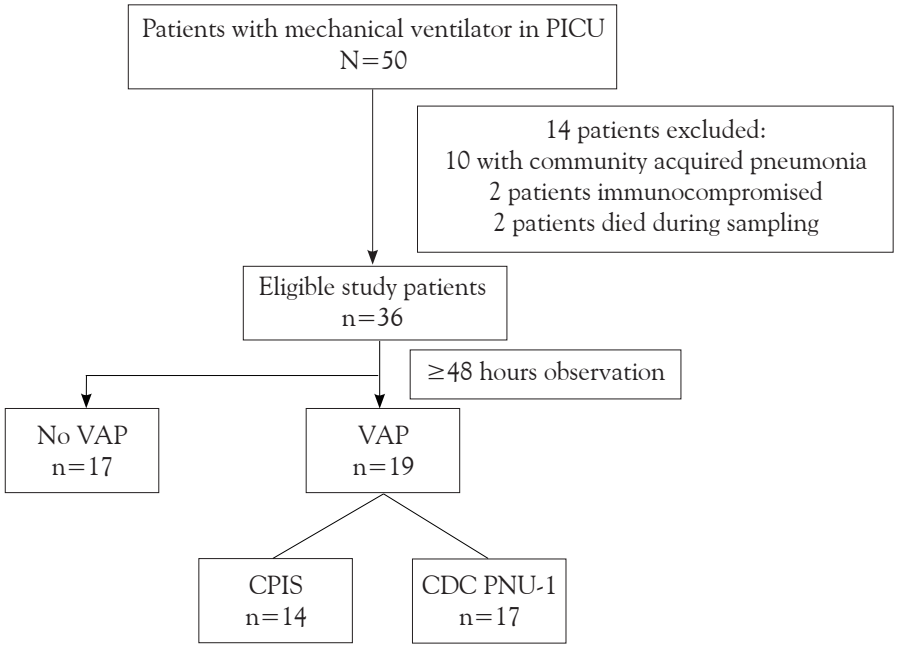

Figure 1. Study flow chart

Table 3. Characteristics of subjects

Characteristics	VAP		P value
	CPIS n=14	CDC PNU-1 n=17	
Gender			
Male	9	9	0.738
Female	5	8	
Mean age (SD), years	3.5 (4.7)		
Nutritional status			
Normal	8	9	0.431
Moderate malnutrition	3	5	
Severe malnutrition	3	3	
Mean PRISM 3 score (SD)	6 (5.2)		
Frequency of intubation			
< 3 times	13	15	0.906
>3 times	1	2	
Difficulty of intubation			
Yes	3	4	0.114
No	11	13	
Mean length of stay (SD), days	14 (15.9)		
Mean duration of ventilation (SD), days	12 (13.7)		
Main Disease			
Neurology	4	5	0.171
Cardiology	4	5	
Nephrology	1	3	
Hematology	2	2	
Gastroenterology	2	1	
Endocrinology	0	0	
Respirology	1	1	
Outcomes			
Survived	9	11	0.813
Died	5	6	

The positive likelihood ratio was 1.12 and negative likelihood ratio was 1.09.

Discussion

Pneumonia is the leading cause of nosocomial infections in the PICU, and the use of mechanical ventilators increases the risk of infection by 6-21 times. As many as 95% of pneumonia cases are nosocomial infections due to VAP, and about 20% die.[10,11] Ventilator-associated pneumonia is associated with increased morbidity, including longer durations of mechanical ventilation and PICU length of stay. Risk factors for developing VAP have been described in multiple studies. A previous study noted increased VAP rates in patients who had experienced witnessed aspiration, reintubation, prior antibiotic therapy, continuous enteral nutrition, and bronchoscopy.[12] Another study found that genetic syndromes, reintubation, and transport out of the PICU were independent predictors of VAP.[13] Srinivasan *et al.*[14] identified enteral nutrition, sedative/narcotic usage, presence of a gastric tube, female sex, prolonged mechanical ventilation, and post-surgical admission as independent risk factors for VAP or healthcare-associated pneumonia in PICUs. Other reported risk factors include immunodeficiency, neuromuscular blockade, blood product usage, or medications such as steroids, H_2 blockers, and metoclopramide.[5]

Guidelines for diagnosis and management of VAP in pediatric patients is currently very limited. Studies comparing assessments with CPIS and post-mortem pathological results showed no significant differences.[15,16] Similarly, a post-mortem study assessed the accuracy of VAP diagnoses by comparing clinical criteria to microbiology (CPIS), and reported sensitivity of 69% and specificity of 72%.[17] Most of researchers agree that pulmonary histological examination coupled with quantitative tissue culture can be an acceptable gold standard, but the method is considered too invasive and difficult for patients on mechanical ventilation.[17]

In developing countries, diagnosing VAP remains a problem, hence, a simple and accurate method is needed. Clinical criteria are still required for early diagnosis of VAP. Biopsy is accurate, but not applicable to small children, so the most widely-used diagnostic tool is CPIS (a gold standard). The Kappa agreement test was performed on data with categorical variables. Both of these criteria have the same ability if the value of agreement between the two variables was high. The kappa value between the CPIS and CDC PNU-1 was 0.61, indicating a good level of agreement. Hence, the CDC-PNU 1 criteria can be used as a diagnostic tool for VAP.

In contrast, Waltrick *et al.*[18] reported that the CDC method could not be used as a surveillance method (kappa value 0.47, sensitivity 37%, and specificity 100%, compared to CPIS). This low sensitivity of the CDC criteria in detecting VAP may have been related to several factors, such as age (subjects were >18 years of age), as well as inability of researchers to observe changes in ventilator settings and clinical changes in all VAP patients, other than the cut-off value. The cut-off CPIS score was also different in their study, at >7. In addition, their inclusion criteria were different as was their treatment protocol which included 30o patient elevation position, gastric ulcer prophylaxis, sedation, and the use of clorhexidin for oral hygiene.

Another study compared CDC surveillance method with CPIS and showed that only 14.5% of cases diagnosed with VAP using CPIS were identified using CDC PNU-1.[19] Their study differed from ours in that they studied adult patients and used a retrospective study design while we diagnosed prospectively in real time. They also had limitations in clinical observation, as they could not identify specific diagnostic criteria when compared with bedside clinical criteria (changes in mental status, purulent secretion), as well as possible false positive data.

In the absence of a gold standard for diagnosing VAP, clinical assessment currently remains important as a substitute. However, Wallace *et al.*[20] found that the system of scoring individuals was poor by assessing VAP clinically using the 2008 CDC-NHSN algorithm, with a low suitability value of $\kappa=0.19$. Their study was not appropriate for evaluating risk factors, because some patients had undergone kidney or bone marrow transplantation, had immunosuppressive diseases or chronic lung disease, which can increase the risk of VAP.

The definition of VAP depends on the integration of clinical findings, as well as radiographic and microbiological data, to make a diagnosis. Clinical

findings can be partially subjective, and therefore, susceptible to variability in documentation and interpretation. In addition, radiographic changes in chest photos can be caused by pathological processes other than pneumonia, or can resemble pneumonia from pulmonary contusions in trauma patients, to pulmonary edema and pleural effusion in heart failure patients. This problem is further complicated by the fact that radiography at times does not detect changes for weeks, potentially masking new processes. A previous study also support the finding that interpretation of chest radiographs can vary between clinicians.[21] The CDC definition of VAP is more subjective and clinical. Although there are many shortcomings such as high subjectivity and low specificity, our findings may have a significant impact if a combination of clinical and objective criteria are evaluated.[21]

Safdar *et al.*[22] studied 73 patients on mechanical ventilators. A total of 36 patients were diagnosed with VAP by the CDC criteria and 35 patients were diagnosed with VAP by the CPIS criteria. They found that the CPIS criteria had very good agreement with the CDC PNU-1 criteria (Cohen's κ 0.81; 95%CI 0.67 to 0.94). Comparison of the CDC criteria to CPIS had sensitivity 0.89 and specificity 0.91, with a positive likelihood ratio of 10.96 and a negative likelihood ratio of 0.12. They used the same exclusion criteria as our study, namely, the exclusion of patients suffering from pneumonia before ventilation and suspected pneumonia during the incubation period while intubated. They also used the same CPIS value cut-off of >6.

The subjects of this study were 14 children suffering from VAP with positive sputum culture results, where the results of the most bacterial culture were *Acinetobacter baumanii* found in 6/36 children. Gadappa *et al.*[23] reported that the most common organisms in early VAP are Acinetobacter baumannii and MRSA, whereas Pseudomonas aeruginosa is the most common organism in late VAP. They noted a no significant association between positive culture and death in VAP (P=0.067). Other studies also reported that Acinetobacter was the most common isolate in VAP.[24-26]

The limitation of this study was that the CDC PNU-1 criteria, while a good early diagnosis tool, did not take into account sputum culture examination, so determination of antibiotic therapy is empirical. Thus, culture examination is still recommended in order to determine the most appropriate subsequent antibiotic therapy.

In conclusion, CDC PNU-1 criteria can be used as an initial diagnostic tool to establish VAP diagnosis, followed by confirmation using other criteria that are close to the gold standard.

Conflict of Interest

None declared.

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Original Article

Levothyroxine use and thyroid gland volumes in children with autoimmune thyroiditis: a systematic review and meta-analysis

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Abstract

Background A hospital-based cancer registry can be used as Background Autoimmune thyroiditis may manifest as overt hypothyroidism, subclinical hypothyroidism, euthyroidism, or hyperthyroidism in children. Although there is no consensus on treating euthyroidism and autoimmune thyroiditis in children, some studies have demonstrated the efficacy of levothyroxine in reducing thyroid volume, improving thyroid function, and stabilizing the immunological process.

Objective To determine the effect of levothyroxine on thyroid gland volume changes, thyroid function, and thyroid antibodies in euthyroid children with autoimmune thyroiditis.

Methods We performed a literature search of electronic databases (the Cochrane Library, *MEDLINE, EBSCO, ProQuest,* clinicaltrials.gov, and other sources, as well as a non-electronic search (searching journals and conference proceedings by hand) to identify studies of euthyroid children with autoimmune thyroiditis published by August 2018. Only English-language articles were included in the search (electronic and non-electronic). Randomized controlled trials that compared levothyroxine with a control (placebo or no treatment) in euthyroid children with autoimmune thyroiditis were selected. The outcome measures were thyroid volume changes, thyroid function, and thyroid antibody levels in euthyroid children with autoimmune thyroiditis. Two authors independently extracted the data, assessed the risk of bias, and analyzed the pooled data from the included studies using a random effects model. The same authors performed a sensitivity analysis.

Results We identified 57 studies. Of these, three studies, involving 97 subjects (51 subjects in an intervention group and 46 subjects in the control group) were selected for inclusion in a systematic review/meta-analysis. The meta-analysis revealed a significant difference in mean thyroid volume changes between the two groups (-1.10 SDs; 95%CI -1.56 to -0.64; I^2=6%; P<0.0001). The mean difference in the thyroid-stimulating hormone (TSH) change of the two groups was -1.82 mU/L (95%CI -3.52 to -0.11; I^2=87%; P=0.04). The standardized mean difference in free thyroxine (fT4) change of the two groups was 0.82 pmol/L (95%CI -1.14 to 2.78; I^2=89%; P=0.41).

Conclusion In euthyroid children with autoimmune thyroiditis, levothyroxine treatment reduces the thyroid volume better. The TSH level change in the intervention group is better than those in the control group. Levothyroxine treatment did not significantly improve free T4. **[Paediatr Indones. 2019;59:202-10; doi: http://dx.doi.org/10.14238/pi59.4.2019.202-10]**.

Keywords: *autoimmune thyroiditis; euthyroid; levothyroxine; thyroid volume*

Autoimmune thyroiditis is a thyroid disorder caused by an autoimmune process. The disease commonly occurs in adolescents, and it is more common in girls than boys

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(ratio: 2:1).[1,2] The incidence of autoimmune thyroiditis is increasing with the annual incidence worldwide is 0.3-1.5 cases per 1000 persons. Multifactorial causes, such as immunological mechanisms, genetics, and the environment are involved in autoimmune thyroiditis.[3,4]

Thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb) are the main antibodies produced in autoimmune thyroiditis.[3] From the clinical history, TgAb are released during the early immune response, but TPOAb are released later.[5] After thyroid antibodies arise, the thyroid glands, maybe affected anatomically and histologically, as a goitrous form or an atrophic form.[6] Autoimmune thyroiditis clinically manifests as euthyroidism (52.1% of cases), overt hypothyroidism (22.2% of cases), subclinical hypothyroidism (19.2% of cases), and occasionally as hyperthyroidism (6.5% of cases).[7,8]

Thyroid function deteriorates along with the progressive increment in antibody levels, especially TPOAb as well as thyroid stimulating hormone (TSH), and enlargement of thyroid gland volume.[2] The aforementioned parameters serve as prognostic markers of the development of hypothyrodism.[2] In autoimmune thyroiditis, ultrasound can be used to measure thyroid volume enlargement and detect lymphocyte infiltration based on echogenicity.[5] An enlarged thyroid gland can persist for a long period in autoimmune thyroiditis. The enlarged thyroid gland commonly increases gradually, but in some cases, it can increase rapidly.[9]

The use of levothyroxine as a treatment for autoimmune thyroiditis in children with hypothyroidism is well established, as is treatment for subclinical hypothyroidism.[2,10,11] However, there is no consensus on the treatment of euthyroid children.[2,5] Some studies reported that levothyroxine treatment in euthyroid individuals with autoimmune thyroiditis reduced thyroid volume changes, as well as TSH and thyroid antibody levels in children with or without goiters.[2,12-19]

Methods

A systematic review of the literature and a meta-analysis were conducted to determine the effect of levothyroxine treatment on thyroid volume changes, thyroid function, and thyroid antibodies in euthyroid children with autoimmune thyroiditis. The inclusion criteria were published and unpublished studies designed as randomized control trials. Children aged 0-18 years with positive thyroid antibodies (antithyroid peroxidase and antibody antithyroglobulin) and normal thyroid function (euthyroid), with or without goiters were included in the study. Patients with other concurrent diseases who met the above inclusion criteria were included. The control (comparison) groups consisted of euthyroid children with autoimmune thyroiditis who received a placebo or no treatment.

The intervention consisted of levothyroxine administration for at least 24 months in euthyroid children with autoimmune thyroiditis. No restrictions were placed on the levothyroxine dose in this study. The outcomes assessed were changes of thyroid volume and changes in the levels of TSH (mU/L), free thyroxine (pmol/L), thyroid peroxidase antibody (TPOAb, U/mL), and thyroglobulin antibody (TgAb, U/mL).

A systematic literature search was conducted in August 2018 to identify studies that met the inclusion criteria. We retrieved only English-language publications and placed no limitation on the year of publication before the time of searching. The following databases were searched: *MEDLINE* (PubMed), the Cochrane Library, *EBSCO*, *ProQuest*, and registered clinical trials (clinicaltrials.gov). In cases where more data from the study of interest was required, an electronic mail was sent to the authors. The potential studies were searched from the citations of clinical trial reports, reviews, meta-analyses, guidelines, and health technology assessments. A non-electronic search (by hand) of abstracts from the *American Thyroid Association and European Thyroid Association* conferences was also performed. The *World Health Organization*'s international clinical trial registry platform (http://www.who.int/ictrp/en/) and clinicaltrials.gov were also searched to identify ongoing clinical trials.

The keywords used were in accordance with medical subject heading (MeSH) terms. Combinations of the following keywords were used: "thyroiditis, autoimmune" "Hashimoto disease," "thyroxine," "euthyroid goiter," (supplementary concept), "euthyroid," "thyroid volume," and "thyroid gland."

Authors independently assessed titles and abstracts to identify potentially relevant articles, taking into account the restriction criteria of this study. The full texts of research articles considered potentially relevant to this systematic review were then appraised to assess their relevance. Any disagreement between the two researchers was resolved by discussion until a consensus was reached. A modified version of PRISMA (preferred reporting items for systematic reviews and meta-analyses) was used for the study selection.[20] A flow chart of the study selection process is presented in **Figure 1**.

The following data were extracted: type of study, study subjects, intervention, and outcomes. Authors also independently assessed the risk of bias in each study based on the Cochrane Collaboration's tool for assessing the risk of bias.[21] The risk of bias consisted of assessment of randomization, allocation concealment, blinding, completeness of the outcome assessment, selective reporting, and other sources of bias.[21] Any disagreement between the two researchers was discussed until a consensus was reached.

The primary outcome in this study was the mean difference in thyroid volume changes, with a 95% confidence interval (CI). The data are presented as standard deviations (SDs). The secondary outcomes were changes in thyroid function and thyroid antibody

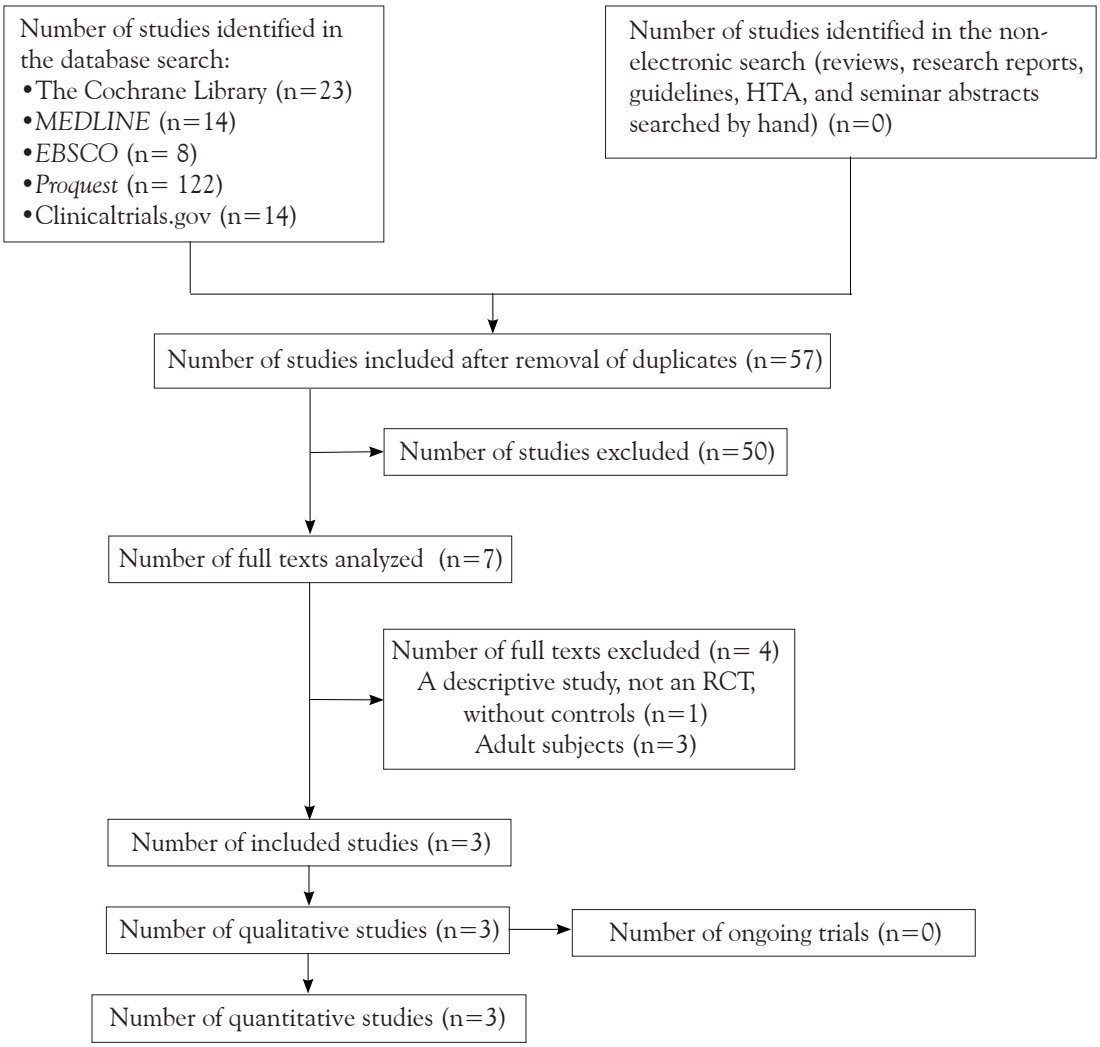


Figure 1. The study flow diagram

levels. Any missing data in the studies were reviewed, and the authors of the studies were contacted. The incidence of drop-outs, lost to follow-up, and withdrawal were reviewed. The missing data were appraised after receipt.

A pooled effect estimation was not obtained for any heterogeneity related to clinical substances, methodology, or statistics. The eyeball test was used to assess heterogeneity in a forest plot, and the Chi^2 test with $\alpha=0.1$ was used to assess significance. The inter-study variation and degree of inconsistency (I^2) in this review were assessed. The degree of inconsistency was categorized in accordance to the Cochrane Systematic Review guidance.[22] A random-effects model was applied if the synthesized studies were heterogeneous, and a fixed-effects model was applied in cases of a low degree of heterogeneity. Due to limited data in the included studies, we did not conduct subgroup analyses.

Results

Fifty-seven studies were identified by the search methods. Of these, 50 studies were excluded based on screening of the abstracts. Following full-text screening of the seven studies, four studies were excluded because they did not meet the inclusion criteria (adult subjects, n=3; not a clinical trial, n=1). The final result included three studies that could be analyzed quantitatively (**Figure 1**). The characteristics of the studies included in this systematic review are shown in **Table 1a** and **Table 1b**.

The quality of the research methods in this systematic review is illustrated in **Figure 2**. There was a low risk of selection bias, detection bias, and reporting bias. All the studies had a randomized control design. The mean difference (SD) in thyroid volume changes was calculated and plotted according to age and sex. However, there was a high risk of performance bias as none of the studies were blinded and none of the controls in the included studies received any treatment (observation only).

The primary outcome of this systematic review was the mean difference (SD) in the thyroid volume change. There was a significant difference in thyroid volume changes in the quantitative analysis (-1.10 SD; 95%CI -1.56 to -0.64; P<0.00001). The meta-analysis indicated that the inconsistency was likely unimportant ($I^2=6\%$; P=0.35) (**Figure 3**). The

Table 1a. The basic characteristics of the studies

Study	Study design (n)	Intervention and control (n)	Intervention duration and dose
Karges *et al.* (2007)[23]	Randomized, open, controlled clinical trial (30)	Levothyroxine (16) *vs.* no treatment (14)	24 months + 6 months observation; 1.3 µg/kg/day
Dörr *et al.* (2015) [24]	Multicenter, randomized, controlled clinical trial (20)	Levothyroxine (10) *vs.* no treatment (10)	36 months; 1.6 ± 0.8 µg/kg/day
Scarpa *et al.* (2010)[25]	Randomized, controlled clinical trial (47)	Levothyroxine (25) *vs.* no treatment (22)	24 months; 1.44 ± 0.5 µg/kg/day

Table 1b. The basic characteristic of the studies

Study	Study population	Study period	Country	Setting	Mean age in the intervention *vs.* control groups (SD), years
Karges *et al.* (2007)[23]	Children with type 1 diabetes mellitus and euthyroid autoimmune thyroiditis	1 September 2002 to 30 December 2003	Germany	Outpatient tertiary care center	12.7 (2.0) *vs.* 13.9 (2.1)
Dörr *et al.* (2015)[24]	Euthyroid children with (goitrous and non-goitrous autoimmune thyroiditis	January 2002 to December 2009	Germany	Six tertiary care centers	10.5 (2.50) *vs.* 13.4 (1.58)
Scarpa *et al.* 2010)[25]	Euthyroid children with non-goitrous autoimmune thyroiditis	January 2001 to October 2005	Greece	Outpatient of pediatric endocrinology clinic in children hospital	12.1 (11.1-13.4) *vs.* 12.2 (11.1-13.0)*

*standard deviation data was not mentioned. Authors used range data.

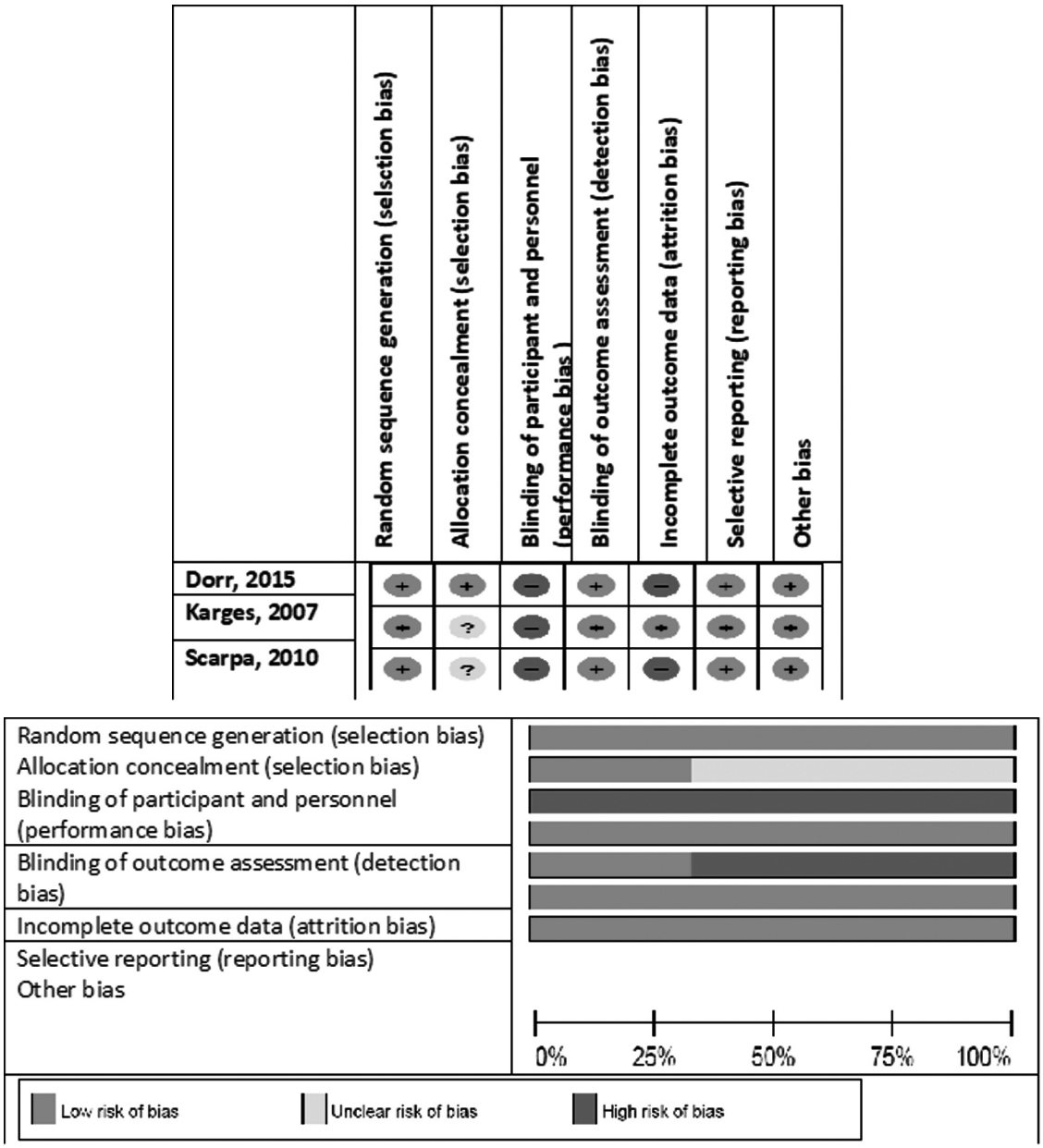
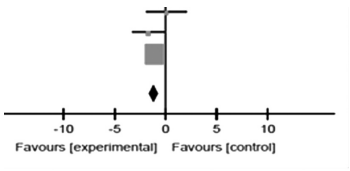


Figure 2. Assessment summary of the risk of bias in the three studies

populations in the studies by Karges *et al.*[23] and Dörr *et al.*[24] included subjects with goiters. In the subgroup analysis of the Karges *et al.*,[23] levothyroxine reduced thyroid volume in the treatment group as compared with that in the controls with goiters (-0.91 SD *vs.* +1.33 SD, P=0.0266). In subjects without goiters, the thyroid volume increased in both groups (intervention vs. control, 0.79 *vs.* 0.83, respectively; but statistically not significant P=0.7922).[23]

The pattern of thyroid volume changes observed differed between groups (**Figure 3**). Dörr *et al*. reported that although thyroid volume changes between the two groups were significantly different during 12-30 months of the study, the mean thyroid volume at the end of the study (36 months) was almost identical in both groups.[24] This observation was due to a tendency toward thyroid volume reductions in the follow-up period (30-36 months)

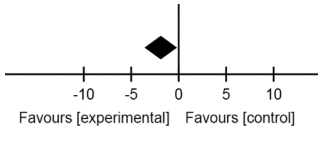
Study	Experimental			Control			Weight	Mean difference, fixed (95%CI), SDs	Mean difference, fixed (95%CI) (SDs)*
	Mean, SDs	SD, SDs	Total	Mean, SDs	SD, SDs	Total			
Dorr, 2015	-0.89	2.66	10	-0.98	1.63	10	5.7%	0.09 (-1.84, 2.02)	
Karges, 2007	-0.60	2.20	16	1.11	1.90	14	9.8%	-1.71 (-3.18, -0.24)	
Scarpa, 2010	-0.50	0.65	25	0.62	1.03	22	84.5%	-1.11 (-1.61, -0.61)	
Total (95%CI)			51			46	100.0%	-1.10 (-1.56, -0.64)	
Heterogenicity: Chi^2=2.12, df=2, I^2=6% (P=0.35)									
Test for everall effect: Z=4.70 (P<0.00001)									

SDs=the thyroid volume; SDs=(measured volume - mean predicted volume for age and sex)/SD; *fixed=fixed effect model

Figure 3. The effect of levothyroxine on thyroid volume changes (SD) in euthyroid children with autoimmune thyroiditis.

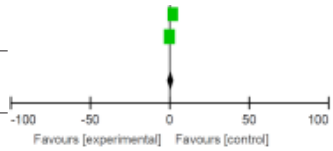
in the control group. Karges *et al.*[23] noted that the thyroid volumes in subjects with goiters decreased in the intervention group and increased in the control group. But in subjects without goiters, the thyroid volumes increased in both groups. Furthermore, Scarpa *et al.*[25] reported that the thyroid volumes were similar in both groups at the 1-year follow-up, but significantly different at the 2-year follow-up. The thyroid volume declined in the intervention group during the 2-year follow-up period, but increased in the control group.[23-25]

The secondary outcomes of this study are described in **Figures 4** and **Figure 5**. The mean difference in TSH levels between the two groups was -1.82 mU/L (95%CI -3.52 to -0.11; I^2=87%; P=0.004). There was a statistically significant different of TSH changes between two groups. The standardized mean difference in free T4 levels between the two groups was an increase of 0.82 (95%CI -1.14 to 2.78; I^2=89%; P=0.41). The mean differences in TPOAb and TgAb levels between the two groups were: 193.53 U/ml (95%CI -201.31 to

Study	Experimental			Control			Weight	Mean difference, random (95%CI), mU/L	Mean difference, fixed (95%CI), mU/L*
	Mean, mU/L	SD, mU/L	Total	Mean, mU/L	SD, mU/L	Total			
Dorr, 2015	-1.81	1.28	10	-0.26	1.73	10	31.5%	-1.55 (-2.88, -0.22)	
Karges, 2007	0.14	1.80	16	0.56	2.40	14	29.7%	-0.42 (-1.96, 1.12)	
Scarpa, 2010	-1.80	0.37	25	1.30	0.49	22	38.8%	-3.10 (-3.35, 2.85)	
Total (95%CI)			51			46	100.0%	-1.82 (-3.52, -0.11)	
Heterogenicity: Tau^2=1.93, Chi^2=15.97, df=2, I^2=87% (P=0.0003)									
Test for overall effect: Z=2.09 (P=0.04)									

*random=random effect model

Figure 4. The effect of levothyroxine on the changes in the level of TSH in euthyroid children with autoimmune thyroiditis

Study	Experimental			Control			Weight	Mean difference, random (95%CI), pmol/L	Mean difference, fixed (95%CI), pmol/L*
	Mean, pmol/L	SD, pmol/L	Total	Mean, pmol/L	SD, pmol/L	Total			
Dorr, 2015	3.80	2.34	10	-0.50	2.07	10	47.85	1.86 90.78, 2.95)	
Karges, 2007	0.07	2.80	16	0.83	7.20	14	52.2%	-0.14 (-0.86. 0.58)	
Total (95%CI)			26			24	100.0%	0.82 (-1.14, 2.78)	
Heterogenicity: Tau^2=1.79, Chi^2=9.07, df=1, I^2=89%(P=0.003)									
Test for overall: effect Z=0.82 (P=0.41)									

*random=random effect model

Figure 5. The effect of levothyroxine on the changes in the level of free T4 in euthyroid children with autoimmune thyroiditis

592.36; I^2=0%; P=0.34) and -93.9 U/mL; (95%CI -265.96 to 78.17; I^2=0%; P=0.28), respectively. There were no significantly different between two groups in free T4 changes, TPOAb changes and TgAb changes.

The side effects reported in the studies are shown in **Table 3**. The incidence of goiter was lower in the intervention group than in the control group (3/25 *vs.* 17/25, respectively) [odds ratio (OR) 0.06; 95%CI 0.01 to 0.28]. Large goiters and goiters with nodules were found only in the control group.25 Levothyroxine administration for 2 years reduced the incidence of hypothyroidism in euthyroid children with autoimmune thyroiditis as compared to controls (3/56 *vs.* 17/53; OR 0.12; 95%CI 0.03 to 0.44).

Discussion

In this systematic review, levothyroxine administration reduced thyroid volume as compared to controls. In all the included studies, levothyroxine treatment for at least 24 months reduced thyroid volume, but thyroid volume increased in the control group.[23-25]

Studies on the natural history of untreated autoimmune thyroiditis reported that thyroid function did not deteriorate in most cases.[7,26,27] In previous studies, the incidence of overt or subclinical hypothyroidism varied (25-43%) in euthyroid subjects.[7,26,27] At present, the use of levothyroxine treatment in euthyroid children remains debatable

Table 3. Side effects of levothyroxine

Study	Intervention (I) and control (C)	Randomized, n	Number of participants upon study completion, n	All side effects, n(%)	Serious/severe side effects, n(%)
Karges *et al.* (2007)[23]	I: levothyroxine	16	16	3 (19)*	0 (0)
	C: no treatment	14	14	4 (29)*	0 (0)
	Total	30	30	7 (23)*	0 (0)
Dörr et al. (2015)24	I: levothyroxine	40	10	0 (0)	0 (0)
	C: no treatment	39	10	13 (33)*	0 (0)
	Total	79	20	13 (16)	0 (0)
Scarpa *et al.* (2010)[25]	I: levothyroxine	25	25	3 (12)** 2 (8)$^{@}$ 0 (0)$^{\&}$	0 (0)
	C: no treatment	25	22	17 (68)** 2 (8)$^{@}$ 2 (8)$^{\&}$	1 (0.04)$^{\#}$ 1 (0.04)$^{\$}$
	Total	50	20 (4)**	4 (8)$^{@}$ 2 (4)$^{\&}$	1 (0.04)$^{\#}$ 1 (0.04)$^{\$}$

*hypothyroidism; **goiter; $^{@}$nodule; $^{\&}$subclinical hypothyroidism; $^{\#}$large goiter; $^{\$}$a goiter and nodule of > 1 cm

due to the potential risk of developing hypothyroidism or goiters.

The mechanism of reducing thyroid volume by levothyroxine treatment is still unclear. According to one theory, it may be due to reduced TSH levels, which decrease antigen expression.[17] This results in decreased lymphocyte infiltration and atrophy of hyperplastic thyroid follicular cells.[17] The mean change in TSH levels in this study supports this theory. Comparing the TSH levels in the treatment and control groups, levothyroxine was beneficial in preventing increments in TSH levels. However, further study is needed to confirm the effect of levothyroxine in individuals with and without goiters.

Quality of life may be impaired in children with autoimmune thyroiditis and goiters. A previous systematic review of untreated autoimmune thyroiditis subjects with thyroid enlargement reported a decrease in overall quality of life (62%), obstacles in daily activities (22-35%), social problems (21%), anxiety disorders (13-60%), and cosmetic complaints (28-36%).[28] This study did not mention the age of the subjects. A study in Italy also reported quality of life impairment (mood disorders, fatigue, and sleep disorders) in euthyroid adults with goiters.[29] To our knowledge, no studies have examined the benefit of thyroid volume improvements in terms of quality of life, especially in children/adolescents.

The TPOAb is an important marker of thyroid function deterioration, as the levels are increased in 95% of autoimmune thyroiditis cases.[2,5] The risk of hypothyroidism increases in accordance with TPOAb level, with higher levels associated with a higher risk.[30] In this study, the changes in TPOAb levels were not different between levothyroxine treatment and control groups. This result was in agreement with other observational studies, but contrary to a study by Korzeniowska et al. who concluded that levothyroxine stabilized TPOAb levels as compared to controls.[15,16,26]

The small number of included studies and subjects were limitations of this meta-analysis. All the included studies also had a high risk of performance bias because of the absence of blinding methods. Another limitation was the composition of the study populations. One study included subjects with and without goiters,[24] and two other studies included only subjects without goiters.[23,25] Furthermore, the duration of the treatment was limited to 24-36 months. Thus, the effect of levothyroxine beyond this period is unknown.

The clinical applicability of this review in non-goiter subjects without any disease or family history of autoimmune thyroiditis was difficult to determine. To date, there are no screening guidelines for healthy individuals with those conditions. The treatment of asymptomatic cases with positive thyroid antibodies needs to be carefully considered, taking into account cost-benefits, psychological aspects of chronic treatment in children, and the natural history of the disease.

In conclusion, there is a significant decrease in thyroid volume in euthyroid children with autoimmune thyroiditis who receive levothyroxine compared to controls. Studies containing larger sample sizes and a double-blinded design are needed to confirm these results.

Conflict of Interest

None declared.

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Original Article

Fluid overload and length of mechanical ventilation in pediatric sepsis

Winda Paramitha, Rina Triasih, Desy Rusmawatiningtyas

Abstract

Background A hospital-based cancer registry can be used as Background Children with sepsis often experience hemodynamic failure and would benefit from fluid resuscitation. On the other hand, critically ill children with sepsis have a higher risk of fluid accumulation due to increased capillary hydrostatic pressure and permeability. Therefore, fluid overload may result in higher morbidity and mortality during pediatric intensive care unit (PICU) hospitalization.

Objective To evaluate the correlation between fluid overload and the length of mechanical ventilation in children with sepsis admitted to the PICU.

Methods Our retrospective cohort study included children aged 1 month-18 years with sepsis who were admitted to the PICU between January 2013 and June 2018 and mechanically-ventilated. Secondary data was extracted from subjects' medical records. Data analyses used were independent T-test and survival analysis.

Results Of 444 children admitted to the PICU, 166 initially met the inclusion criteria. Of those, 17 children were excluded due to congenital heart disease. Subjects' median age was 19 months and median PELOD-2 score was 8. Eighteen children (12.1%) had positive fluid balance in the first 48 hours. Median mechanical ventilation duration was 5 days. Fluid overload was significantly correlated with length of mechanical ventilation (P=0.01) and PICU mortality (RR=2.06; 95%CI 2.56 to 166; P=0.001). Neither length of PICU stay nor extubation failure were significantly correlated to fluid overload.

Conclusion Fluid overload was significantly correlated with length of mechanical ventilation and may be a predictor of mortality in children with sepsis in the PICU. **[Paediatr Indones. 2019;59:211-6; doi: http://dx.doi.org/10.14238/pi59.4.2019.211-6]**.

Keywords: *fluid overload; sepsis; length of mechanical ventilation*

Sepsis is a life-threatening clinical condition frequently faced by PICU pediatricians.[1] The prevalence of sepsis was reported to be 8.2% globally in 2013, with a 15.3% prevalence in Asia.[2,3] Therapeutic approaches to sepsis are comprised of resucitation (including fluid), pathogen eradication, maintaining oxygen delivery, and inflammatory response modification.[4] Increased capillary permeability and changes in oncotic gradient pressure are pitfalls in sepsis management that lead to fluid overload.[5,6] Therefore, fluid administration to sepsis patients either for resucitation or maintenance necessitates careful attention by the physician. Fluid may accumulate in various organs, inducing organ dysfunction. Fluid accumulation in the lung interstitium and alveoli may induce lung edema and ventilation perfusion mismatch. In patients on mechanical ventilation, these conditions may result in prolonged ventilation. Previous studies by Vidal *et al.*[7] and Arikan *et al.*[8] found that fluid overload could extend the length of mechanical ventilation and PICU stay. Fluid overload was also related to

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increased mortality rate in adult sepsis.[9] Hence, we aimed to evaluate for a possible correlation between fluid overload and mechanical ventilation duration in a pediatric sepsis population.

Methods

We conducted a retrospective cohort study and reviewed all children admitted to the PICU of Dr. Sardjito Hospital, Yogyakarta, from January 2013 to June 2018. Children with sepsis who required mechanical ventilation (MV) within the first 24 hours of care and were hospitalized in the PICU for more than 48 hours were included in the study. Those with brainstem death, end-stage renal disease on routine hemodialysis, suspected or confirmed congenital heart disease, or whole body (anasarca) edema were excluded. Subjects were included using consecutive sampling and data regarding basic characteristics, fluid balance, length of MV, length of PICU stay, and mortality were collected from medical records. All children's medical records were reassessed using the Indonesian Pediatric Society criteria for sepsis.[10] The primary outcome in our study was fluid overload percentage (%FO), defined as percentage of fluid balance (difference between fluid input and output) in the first 48 hours divided by body weight on admission. Secondary outcomes were PICU mortality and length of PICU stay. Data analyses were performed with *SPSS 24* software.

This study was approved by the Medical Ethics Committee at Universitas Gadjah Mada.

Results

A total of 444 children with sepsis required mechanical ventilation from January 2013 to June 2018. One hundred and sixty six children met the inclusion

Figure 1. Study plot and sample collection steps

criteria upon initial screening. Of these, 17 children were excluded due to echocardiographically-confirmed congenital heart disease (**Figure 1**). We calculated the first 48 hours cumulative fluid balance and %FO based on fluid administration data and admission weight in subjects' medical records. Basic characteristics of subjects are presented in **Table 1**. Subjects' median age was 19 months, with males predominating (59.7%). Underlying diseases were classified into 9 categories as shown in **Table 1**. Of 33 patients with acute kidney injury, 22 (67%) died. Nevertheless, acute kidney injury was not significantly correlated with mortality in our study (P=0.051). Seventy-six (51%) subjects had chronic illness comorbidities and 45 (59.2)% of them died.

Table 1. Basic characteristics of subjects

Characteristics	(N=149)
Median age (IQR), months	19 (7-84.5)
Median body weight (IQR), kg	9.3 (6.9-18.5)
Median PELOD-2 score (IQR),	8 (7-9)
Male sex, n (%)	89 (59.7)
Underlying disease, n (%)	
Respiratory	54 (36.2)
Neurologic	35 (23.5)
Gastrointestinal	3 (2.0)
Tropical disease	26 (17.4)
Hematooncologic	8 (5.4)
Cardiovascular	2 (1,3)
Immunological	5 (3.4)
Surgical	14 (9.4)
Others	2 (1.3)
Acute kidney injury, n (%)	33 (22.1)
Chronic illness, n (%)	76 (51)

Table 2 presents the outcomes of our study. Many more subjects had <10% FO (131 subjects; 87.9%) than ≥ 10% FO (18 subjects; 12.1%). Median length of mechanical ventilation was 5 days, with the longest duration of 60 days. More than half the patients died in the PICU (77 subjects; 51.7%), mostly from septic shock.

Independent T-test revealed no significant mean difference in mechanical ventilation duration between the two %FO groups (P=0.85) (**Table 3**). Other factors such as age, PELOD-2 score, and types of underlying disease also did not significantly correlate with mechanical ventilation duration.

Survival analysis was performed to analyze the relationship between %FO value and length of mechanical ventilation (**Figure 2**). Subjects who eventually died and those whose actual duration of MV could not be calculated were censored. Mean duration of MV in the ≥ 10% FO group was longer than that of the <10% FO group (56.72 *vs.* 13.41 days) and log rank test revealed a significant mean difference (P=0.01). In the ≥ 10%

Table 2. Outcomes of the study

	(N=149)
% FO	
FO <10%, n (%)	131 (87.9)
FO ≥ 10% body weight, n (%)	18 (12.1)
Median duration of MV, days (IQR)	5 (3.5-9)
Median length of PICU stay, days (IQR)	7 (5-13.5)
Mortality in the PICU, n (%)	77 (51.7)

Table 3. Bivariate analysis of %FO and length of mechanical ventilation

	Mean length of mechanical ventilation, days (SD)	95%CI	P value
% FO		-4.01 to 3.28	0.85
≥ 10%	8 (13)		
< 10%	8 (6)		
Age		-0.50 to 5.16	0.11
< 12 months	9 (10)		
≥12 months	7 (5)		
PELOD-2 score	8 (7)		0.49
Underlying diseases			0.411
Respiratory	9 (9)		
Neurologic	8 (5)		
Gastrointestinal	7 (1)		
Tropical disease	6 (4)		
Hematooncologic	5 (3)		
Cardiovascular	6 (2)		
Immunological	6 (3)		
Surgical	11(10)		
Others	7 (6)		

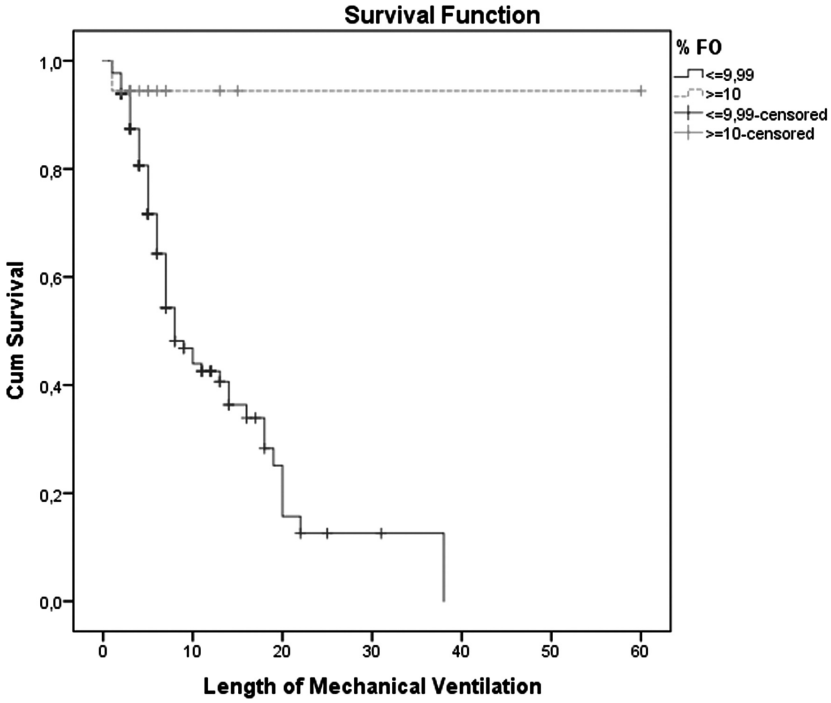

Figure 2. Survival analysis of %FO and MV duration

Table 4. Bivariate analysis of %FO and length of PICU stay

	Mean length of PICU stay, days (SD)	95%CI	P value
% FO		-3.71 to 6.20	0.62
≥ 10%	10 (17)		
< 10%	11 (9)		
Age		0.08 to 6.26	0.04
< 12 months	13 (11)		
≥12 months	9 (8)		
PELOD-2 score	11 (9)		0.46
Underlying diseases			0.52
Respiratory	12 (11)		
Neurologic	11 (10)		
Gastrointestinal	12 (20		
Tropical disease	9 (5)		
Hematooncologic	5 (3)		
Cardiovascular	6 (2)		
Immunological	6 (3)		
Surgical	13 (13)		
Others	11 (11)		

FO group, all but one subject died in the PICU, therefore, the actual total MV duration could not be calculated.

Table 4 shows the bivariate analysis results of %FO and length of PICU stay. Age of <12 months was the only factor significantly correlated to length of PICU stay (P=0.04).

Table 5 presents the whole PICU mortality among subjects based on fluid status, age, PELOD-2 score and underlying disease. Mortality was significantly higher in the ≥ 10% FO group and affected by type of underlying disease. However, which underlying disease that mostly contribute to the mortality could not be furtherly determined.

Table 5. Bivariate analysis of %FO and PICU mortality

	Non-survivor (n=77)	Survivor (n=72)	RR	95%CI	P value
% FO, n(%)			2.06	1.66 to 2.56	0.001
≥ 10%	17 (22)	1 (1.4)			
< 10%	60 (78)	71 (98.6)			
Age, n(%)			0.85	0.61 to 1.19	0.32
< 12 months	26 (33.8)	30 (41.7)			
≥12 months	51 (66.2)	42 (58.3)			
Mean PELOD-2 score (SD)	9 (2)	9 (2)		-0.68 to 0.71	0.96
Underlying diseases					
Respiratory	26 (33.7)	28 (38.9)			0.009
Neurologic	18 (23.4)	17 ((23.6)			
Gastrointestinal	0 (0)	3 (4.2)			
Tropical disease	8 (10.4)	18 (25.0)			
Hematooncologic	6 (7.8)	2 (2.8)			
Cardiovascular	2 (2.6)	0 (0)			
Immunological	5 (6.5)	0 (0)			
Surgical	10 (13.0)	4 (5.5)			
Others	2 (2.6)	0 (0)			

Discussion

Patients in the PICU are susceptible to fluid overload following intravenous fluid administration or deterioration during critical illness. Sepsis and multiple organ failure increase the risk of fluid overload-related complications due to glycocalyx breakdown and pre-existing organ dysfunction.[5]

We studied children aged 1 month to 18 years with sepsis and who were mechanically ventilated. Their median age was 19 months, with male predominance (89 males; 59.7%). The main underlying diseases found in our subjects were respiratory, especially community-acquired pneumonia (36.2%), followed by neurological problems and tropical infections (23.5% and 17.4%, respectively). The respiratory system is the most common infection site found in pediatric sepsis globally.[2] A total of 76 (51%) subjects had chronic illness comorbidities, of whom 59.2% eventually died. Unsurprisingly, previous study by Hartman et al. also figured out that septic children with pre-existing chronic illness had higher risk of mortality compared to previously healthy children (10.5% vs. 8.1%, respectively; P<0.001).[1]

Survival analysis revealed a significant mean difference in mechanical ventilation duration between the two %FO groups, with log rank P value of 0.01 (**Figure 2**). The longest duration of mechanical ventilation was in the ≥10% FO group. This finding was consistent with previous study witheld by Arikan *et al.*[8] which showed that fluid overload was independently correlated with longer duration of MV in children with critical illness (HR 0.95; 95%CI 0.92 to 0.98; P=0.004). Other factors such as age, PELOD-2 score, and underlying disease had no significant correlations with MV duration.

A previous study reported a significant correlation between fluid overload in the first 24 hours of care and mortality in 202 subjects (OR 1.22; 95%CI 1.12 to 1.33; P<0.001).[11] Fluid overload of ≥ 10% was also significantly predictive of mortality in our subjects. Although the length of PICU stay seemed shorter in ≥10% FO group, independent T-test revealed no significant difference between the 2% FO groups. On the contrary, Arikan et al. found that fluid overload >15% was strongly related with longer PICU stay.[8] Koonrangsesomboon *et al.*[12] also found that mean fluid balance in the first 72 hours after septic shock was independently related to ICU length of stay and mortality. In our study, age was the only independent factor predictive of length of PICU stay, with longer duration in the <12-month age group. This finding was consistent with a study by Nupen *et al.*[13] which found that younger age was significantly related to longer duration of PICU stay (P=0.03).

Various studies have been performed to identify correlations between fluid overload and length of mechanical ventilation in critically ill patients.[7,8] Our study

measured similar variables in a more specific population, i.e., critically ill patients who were clinically proven to have sepsis. The limited sample size and risk of inaccurate recording of fluid input and output during PICU admission bias were limitations of our study. However, all fluid administration data collected from medical records were recalculated to minimize any risk of bias. In conclusion, fluid overload in pediatric sepsis significantly correlates with length of mechanical ventilation and PICU mortality, but not with length of PICU stay.

Conflict of Interest

None declared.

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Original Article

Vascular endothelial growth factor (VEGF) expression in induction phase chemotherapy of acute lymphoblastic leukemia

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Abstract

Background Leukemia is a hematolymphoid malignancy originating from bone marrow. The progression of hematolymphoid malignancies depends on new formation of vasculature, called angiogenesis. Angiogenesis is regulated by vascular endothelial growth factor (VEGF), which is secreted by paracrine and autocrine signaling mechanisms.

Objective To evaluate VEGF expression in induction phase chemotherapy of acute lymphoblastic leukemia (ALL) patients.

Methods This prospective, cohort study was conducted in ALL patients admitted to Dr. Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, from October 2016 to October 2017. Subjects' VEGF levels were measured at diagnosis and at the end of induction chemotherapy.

Results VEGF levels were analyzed in 59 patients, 29 of whom were diagnosed with standard risk ALL and 30 patients with high risk ALL. VEGF levels were significantly decreased after induction phase chemotherapy in standard risk ALL and in high risk ALL subjects. There was no significant difference in VEGF levels before induction phase chemotherapy between the standard and high risk groups (P=0.405). There was also no significant difference in VEGF levels after induction phase chemotherapy between the two risk groups (P=0.094).

Conclusion The VEGF level is significantly lower after ALL induction phase chemotherapy in both the standard risk and high risk ALL groups. However, there are no significant differences in VEGF levels between the standard and high risk groups before as well as after induction phase chemotherapy. **[Paediatr Indones. 2019;59:217-21; doi: http://dx.doi.org/10.14238/pi59.4.2019.217-21]**.

Keywords: *angiogenesis; acute lymphoblastic leukemia; VEGF*

Acute leukemia is defined as a blood cell malignancy originating from the bone marrow, and characterized by proliferation of white blood cells with abnormal cell manifestations in peripheral blood and extramedullary sites. Leukocytes in the bone marrow proliferate irregularly and uncontrollably. In addition, leukocyte function becomes abnormal. Because of this process, other functions of normal blood cells are also disrupted, causing leukemia symptoms.[1,2] Acute leukemia in children comprises approximately 30-40% of malignancies in children, and can occur at all ages. The highest incidence generally occurs at the age of 2-5 years, with an average incidence of 4-4.5 cases/year/100,000 children under the age of 15. Although the exact cause of acute lymphoblastic leukemia is still unknown, several factors are involved, including

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endogenous and exogenous exposure (to ionizing radiation, pesticides certain solvents or viruses), genetic involvement, and risk factors.[1,3]

Angiogenesis is the process of capillary formation of blood vessels which is important in wound healing, development, reproduction, and hematological malignancy. The VEGF over-expression is associated with tumor growth, invasion, and metastasis in malignancy, especially with regards to solid tumors. The growth and survival of cancer cells are influenced by supply of oxygen, nutrients, and VEGF by endothelial cells in angiogenesis. Previous studies observed that tumor growth can be suppressed by inhibition receptor of VEGF, therefore no angiogenesis signal is produced.[3] Vascular endothelial growth factor (VEGF) is a heparin-binding, homodimeric glycoprotein or ligand with five components: VEGF-A (or VEGF) with several isoforms, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PIGF). The VEGF shows target cell specificity to endothelial cells. Some cell types, including fibroblasts, endothelial cells, and keratinocytes produce VEGF in small amounts.[4,5]

The progression of hematolymphoid malignancies depends on the induction of new blood vessel formation, such as in acute leukemia, myelodysplasia syndrome, multiple myeloma, and lymphoma.[6] The VEGF is the most important proangiogenic agent. It activates receptors in vascular endothelial cells and increases blood vessel regeneration.[7] The angiogenesis process begins with the release and formation of angiogenic growth factors that diffuse around the damaged tissue. This angiogenic growth factor then binds to specific endothelial cell receptors in the nearby blood vessels and signals growth from the cellular surface to be transmitted to the nucleus. Furthermore, endothelial cells produce new molecules including various enzymes that dissolve proteins and form small holes in the basement membrane to proliferate.[3]

The survival of cancer cells depends heavily on nutrient and oxygen intake. Such cancer cells develop angiogenesis through VEGF production. The VEGF can be produced by the tumor cells themselves, to act through paracrine or autocrine signalling mechanisms. In addition, endothelial cells produce normal VEGF and have receptors that bind to VEGF (VEGF-R2). The main mediators of tumor angiogenesis are specifically the VEGF 121 and 165 isoforms. These isoform signals pass through the VEGF 2 receptor (VEGFR-2), the main receptor mediating the process of angiogenesis. Many cancer cells express VEGF, and increased tumor expression is often associated with a poor prognosis.[8] The VEGF signals mainly through VEGF receptor 2 (VEGFR-2), which is expressed at elevated levels by endothelial cells engaged in angiogenesis and by circulating bone marrow-derived endothelial progenitor cells. The detailed structure of VEGFR-2 in endothelial cells is unknown, but receptors are usually described as functioning to bind signal molecules and located on cellular surfaces. Chemotherapy is beneficial for treatment of hematological malignancies, as it destroys malignant cells, decreasing production of VEGF.[9] Therefore, it is important to know the VEGF levels before and after induction phase chemotherapy. Kalra *et al*.[6] showed that VEGF levels were higher in relapse than at diagnosis, but such studies have been limited. Since VEGF plays a role in angiogenesis to supply cancer cell requirements, leukemia patients are at risk of cachexia. Moreover, decreased VEGF levels produced by cancer cells after chemotherapy, are an indication of good response to treatment. However, VEGF levels that do not change or remain high indicate that leukemia cells are still proliferating and/or the patient is at risk of relapse.[9]

This study aimed to evaluate VEGF expression in induction phase chemotherapy of acute lymphoblastic leukemia (ALL) patients.

Methods

This prospective cohort study was conducted in DR Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, during October 2016-October 2017. VEGF levels were compared before and after induction phase chemotherapy in patients with acute lymphoblastic leukemia (ALL). Subjects' parents provided informed consent for study inclusion. The Ethics Commission of Hasanuddin University and Dr. Wahidin Sudirohusodo Hospital approved the study.

Subjects were patients with an ALL diagnosis aged 1 month to 18 years. Twenty-one patients who did not complete chemotherapy were excluded (15 died, 2 refused chemotherapy, and 4 dropped out of chemotherapy).

Patients aged 1 month - 18 years were diagnosed based on clinical symptoms and the results of complete blood examination and bone marrow puncture (BMP). Data recorded were age, sex, weight, body length/height, nutritional status, vital signs (temperature, pulse, blood pressure, breathing, and consciousness), and routine blood tests. There were classified according to the risk into two groups: a standard-risk group (SR) and a high-risk group (HR). Inclusion criterias for high risk ALL: age < 1 year or > 10 years, leucocyte ≥ 50,000 cells/mm^3 of blood, > 5 leukemic cells in CSF, testicular involvement, mediastinal mass, and leukemia cells with chromosome changes. The standard risk ALL inclusion criterias : age 1-10 years, leucocyte < 50,000 cells/mm^3, < 5 leukemic cells in CSF, no testicular involvement, no mediastinal mass, and no chromosome changes in leukemia cells. Blood specimens were taken at Day 1 before induction phase and at Day 42/44 after induction phase of chemotherapy for VEGF measurements. Serum was separated and stored at -80° C to avoid loss of bioactive human VEGF. The Assay Designs Human VEGF Enzyme Immunometric /assay kit was used to assess serum consentration of VEGF.

Data were grouped based on the purpose and type into the appropriate ALL group, as well as before and after chemotherapy. Univariate analysis, bivariate analysis, and correlation test were used for statistical analyses.

Results

Of 80 patients aged 1 month to 18 years diagnosed with ALL, 59 patients met the inclusion criteria, including 29 patients with standard risk (SR) and 30 patients with high risk (HR). The SR group consisted of 16/29 males while the HR group consisted of 20/30 males. The ALL SR subjects were aged 1-10 years, while the HR subjects were mostly aged 1-10 years (17/30), followed by ≥ 10 years (12/30), and <1 year (1/30). Nutritional status of the SR group consisted of 14/29 well-nourished subjects, 8/29 undernourished subjects, and 7/29 malnourished subjects; the HR group had 17/30 well-nourished subjects, 4/30 undernourished subjects, 7/30 malnourished subjects, and 2/30 overweight subjects (**Table 1**).

Median VEGF levels in the standard risk ALL group were 1,686 (range 217-14,879) ng/L before chemotherapy and 971 (range 450-2,880) ng/L after chemotherapy. Wilcoxon test revealed a significant difference in VEGF levels before and after chemotherapy (P=0.000) (**Table 2**).

Median VEGF levels in the high risk ALL group level were 2,866.5 (range 92-16,452) ng/L before chemotherapy and 1,238 (range 702-2,698) ng/L after chemotherapy. Wilcoxon test revealed a significant difference in VEGF levels before and after chemotherapy in the HR group (P=0.001) (**Table 3**).

Wilcoxon test revealed no significant difference in VEGF levels before chemotherapy between the SR and HR groups (P=0.405). In addition, Wilcoxon test

Table 1. Characteristics of subjects

Characteristics	Acute lymphoblastic leukemia	
	Standard risk (n=29)	High risk (n=30)
Sex		
Male	16	20
Female	13	10
Nutritional status		
Well-nourished	14	17
Undernourished	8	4
Malnourished	7	7
Overweight	0	2
Age		
< 1 year	0	1
1- 10 years	29	17
>10 years	0	12

Table 2. Comparison of VEGF levels before and after chemotherapy in SR patients

VEGF level, ng/L	Standard risk		P value
	Before chemotherapy	After chemotherapy	
Mean (SD)	3,206.8 (3,901.3)	1,154.3 (477.2)	0.000
Median (range)	1,686 (217-14,879)	971 (450-2,880)	

Wilcoxon test (P<0.05)

Table 3. Comparison of VEGF levels before and after chemotherapy in HR patients

VEGF level, ng/L	High risk		P value
	Before chemotherapy	After chemotherapy	
Mean (SD)	3,377.3 (3365.6)	1,398 (555.1)	0.001
Median (range)	2,886.5 (92-16,452)	1,238 (702-2,698)	

Wilcoxon test (P<0.05)

Table 4. Comparison of VEGF levels between the SR and HR groups before and after chemotherapy

VEGF level, ng/L	Before chemotherapy		P value	After chemotherapy		P value
	Standard risk	High risk		Standard risk	High risk	
Mean (SD)	3,206.8 (3,901.3)	3,377.3 (3,365.6)	0.405	1,154.1 (477.2)	1,398 (555.1)	0.094
Median (range)	1,686 (217-14,879)	2,886.5 (92-16,452)		971 (450-2,880)	1,238 (702-2,698)	

Wilcoxon test (P>0.05)

revealed no significant difference in VEGF levels after chemotherapy between the two groups (P=0.094) (**Table 4**).

Discussion

The VEGF levels were significantly reduced in SR and HR ALL patients after induction phase chemotherapy. This finding may suggest that chemotherapy has an anti-angiogenic effect in these patients.

We should note that the authors did not look at vascularization before and after. Angiogenesis is the process of forming new capillaries from blood vessels and is an important process in development, wound healing, and reproduction. It is also an important factor in hematological malignancies, including acute and chronic leukemia, multiple myeloma, and myelodysplasia syndrome. Excessive expression of VEGF is associated with tumor growth, invasion, and metastasis in malignancies, especially with regards to solid tumors.[2] The growth and survival of cancer cells are influenced by supply of oxygen, nutrients, and VEGF by endothelial cells in angiogenesis.[3] The key, pro-angiogenic mediator of the angiogenesis process is VEGF. The VEGF is a glycoprotein that stimulates angiogenesis and capillary permeability by binding to tyrosine kinase receptors 1 and 2. Many leukemias are associated with angiogenesis, since HL60 cells from acute myeloid leukemia are used for gene clones.[3]

Acute lymphoblastic leukemia management is divided into standard risk and high risk groups. Current conventional chemotherapy is used as an antiangiogenic therapy for leukemia cells. Antiangiogenic therapy used as monotherapy has proven to have no effect or benefit in the treatment of hematological malignancies so far. However, some antiangiogenic therapies combined with conventional chemotherapy have been shown to be effective in suppressing the angiogenesis process.[8]

Of 59 subjects in our study, 29 subjects were categorized to have standard risk ALL, with a male: female ratio of 1.3: 1, and 30 subjects were categorized to have high risk ALL with a male: female ratio of 2: 1. Kamima *et al.*[11] in Cipto Mangunkusumo Hospital reported a similar male: female ratio of 1.3: 1 of ALL patients.[10] Also, Silawati in Wahidin Sudirohusodo Hospital reported a ratio of 1.5: 1.

Malnutrition has an impact on multifactorial prognosis. Variance of body composition of lean body mass and adipose tissue will affect the pharmacokinetic and pharmacodnamic of many drugs we use. Previous studies showed that nutritional status should be considered by treating pediatric oncologist.[12] In our study most subjects with standard risk ALL were well-nourished, followed by undernourished, and malnourished. High risk ALL subjects were also mostly well-nourished, while the rest were undernourished, malnourished, and overweight. However, in our study we did not analyze correlation between nutritional status and prognosis.

Angiogenesis has proven to be an important process in hematological malignancies. Several studies have reported an association between leukemia and angiogenesis, since HL60 cells, the acute myeloid leukemia cell line, are used as clones of the VEGF gene. Increased bone marrow vascularization has been found in acute and chronic leukemia in both adults and children.[13]

Median VEGF level in standard risk protocol patients was significantly decreased after chemotherapy compared to before chemotherapy. Leblebisatan *et al.*[14] found that VEGF was higher in leukemia subjects than in healthy subjects. In addition, VEGF levels were not significantly different between AML and ALL patients, but VEGF levels at remission were lower than at the time of initial diagnosis.[12] Also, Giles15 noted that VEGF levels in 417 patients with acute myeloid

leukemia (AML) and myelodysplastic syndromes (MDS) were higher compared to controls.

Median VEGF level in high risk protocol patients was significantly decreased after chemotherapy compared to before chemotherapy. The decreased VEGF levels after chemotherapy may suggest that chemotherapy successfully suppresses angiogenesis in leukemia. Conventional chemotherapy was used as an antiangiogenic therapy for leukemia cells. However, some antiangiogenic therapies combined with conventional chemotherapy have been effective in suppressing the angiogenesis process.[9]

The weakness of this study was that we only measured VEGF level without considering other anti-angiogenesis effects. The strength of this study was its prospective cohort design which is useful for determining the course of the disease or the effects studied in explaining the risk dynamics and outcome effects. Our findings also provide data on VEGF levels in both standard risk and high risk ALL patients. Thus, reference VEGF levels may be useful in future studies on possible associations between leukemia, angiogenesis, and chemotherapy. Further studies on association between VEGF and remission/relapse are required. Multi-center or meta-analysis studies in other hospitals in Indonesia need to be done in order to validate our findings.

In conclusion, VEGF levels significantly decrease after chemotherapy, both in standard risk and high risk ALL patients. There is no significant difference in VEGF levels in standard risk and high risk ALL patients before chemotherapy as well as after chemotherapy.

Conflict of Interest

None declared.

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Case Report

Acute hematogenous osteomyelitis in children: a case series

Komang Agung Irianto[1], Adhinanda Gema[1], William Putera Sukmajaya[2]

Abstract

Background Chronic osteomyelitis is still a major cause of morbidity and disability in children living in developing countries. Neglect of acute osteomyelitis and its progression to chronic osteomyelitis leads to significant morbidity. This report is the first series to describe such cases in Indonesia.

Objective To describe 12 pediatric cases of chronic osteomyelitis in order to remind clinicians about the debilitating complications of musculoskeletal infection.

Methods This report is a case series of 12 children with chronic osteomyelitis admitted to Dr. Soetomo General Hospital, Surabaya, East Java, in 2011-2017. We acquired data from medical records. The patients' quality of life was measured using the Child Health Assessment Questionnaire Disability Index (C-HAQ-DI).

Results The patients' mean age was eight years and they were predominantly male. The most common infection location was the femur (7/12). Microbial cultures were positive in 9/12 of cases, predominantly with Staphylococcus aureus. Erythrocyte sedimentation rate (ESR) was elevated in 11 patients. All patients were diagnosed late, with an average delay of presentation to Orthopedics of 10.5 months. Most of patients experienced mild to moderate disability after the disease, as assessed by the C-HAQ-DI.

Conclusion Diagnosis of osteomyelitis in children is quite difficult, given the lack of specific diagnostic tests. Delayed diagnosis and inappropriate treatment may result in long-term morbidity and disability. Clinicians should have an increased awareness of the clinical features of osteomyelitis, including unusual presentations such as calcaneal osteomyelitis. **[Paediatr Indones. 2019;59:222-8; doi: http://dx.doi.org/10.14238/pi59.4.2019.222-8]**.

Keywords: *chronic hematogenous osteomyelitis; diagnosis pitfall; morbidity; pediatric; C-HAQ-DI*

Osteomyelitis is an infection involving the bone, bone marrow, periosteum, and surrounding soft tissues, resulting in sequestrum and destruction of the bone. The clinical course of chronic osteomyelitis is varied with intermittent pattern and high rate of recurrence. Moreover, complete cure is difficult to achieve.[1]

The symptoms of acute osteomyelitis typically appear within two weeks after bacterial infection. Chronic osteomyelitis may progress six weeks after initial infection, and is marked by the presence of sequestrum.[2] Pediatric chronic osteomyelitis is currently rare in developed countries, but remains a burden in third-world countries. This disease poses severe morbidities in children for the remainder of their lives.[3] In Cambodia, the incidence of pediatric musculoskeletal infection was 13.8/100,000, of which 51% was single-limb osteomyelitis.[4] Meanwhile, in the Philippines, the prevalence of osteomyelitis was 0.015% of total children.[5] However, the epidemiological data regarding musculoskeletal

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infection, especially chronic osteomyelitis, are still limited in Indonesia.

In recent years, Indonesia experienced a health reformation which has increased overall health coverage in citizens. However, there is still a significant disparity between urban and rural areas. As such, the problems of malnutrition and infection in children remain unsolved.[6] Poverty is one of the obstacles for the patients with chronic osteomyelitis to obtain treatment.[7]

Here we describe a series of pediatric chronic osteomyelitis cases, the course of the disease, diagnosis pitfalls, treatments, and outcomes. This report was done in order to remind clinicians about the debilitating outcomes caused by late diagnosis and treatment of musculoskeletal infections.

Methods

This case series included children diagnosed with chronic hematogenous osteomyelitis and admitted to Dr. Soetomo Hospital, Surabaya, East Java, from January 2011 to December 2017. Data were acquired from the electronic medical records. The patients' medical and surgical records, microbial culture results, and radiographs were all reviewed. The data collected were demographic data, anatomical location of infection, length of time before presenting to an orthopedic surgeon, microbial culture result, treatment, and morbidity. Length of time before presenting to an orthopedic surgeon was defined to any delay before presenting to orthopedics after initial contact to any healthcare professionals. Patients' quality of life was measured using the C-HAQ-DI.[8] Diagnosis was based on the patient history and physical examination. Laboratory values, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and complete blood count (CBC), were obtained to aid in the diagnosis. Radiographic evaluation included plain radiographs of the affected area.

Patients were interviewed either in person or over the phone to assess their current condition in terms of quality of life. The C-HAQ-DI index values were categorized as mild to moderate disability (score 0 to 1); moderate to severe disability (score 1 to 2); or severe to very severe disability (score 2 to 3).

Results

Twelve pediatric patients with chronic hematogenous osteomyelitis were reviewed (seven males and five females). The patients' mean age was eight years, with a range of 2-13 years. In the course of disease, destruction of the affected bone was progressive, as depicted in one patient (**Figure 1**).

All patients had some degree of morbidity, which affected their ambulation. The most common complaint was pain (3/12), followed by swelling (3/12). One patient presented with a pathological fracture. The predominant anatomical locations were the femur (7/12) and tibia (3/12); other sites were the calcaneus and humerus.

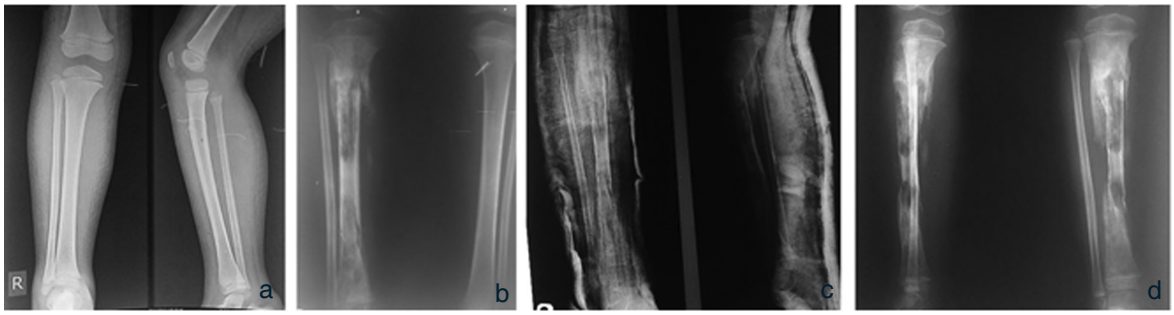

Figure 1(a) X ray of osteomyelitis patient one week after onset of the disease, there is no bone destruction, only tissue swelling. (b) (c) (d) Radiological appearance of patient with neglected acute hematogenous osteomyelitis of right tibia (different patient from figure a), showing the progressive destruction of the bone within a year; figure (b) was taken two months after onset of the disease; figure (c) was taken two months after onset of the disease, directly after surgery; and figure (d) was taken three months after onset of the disease.

Most patients had visited a health care facility (9/12), and only one patient had gone to a traditional bonesetter. The average time for patients to present to an orthopedic surgeon was 10.5 months. Seven patients visited general practitioners and one patient went to a pediatrician. The longest interval was three years and the shortest was 1.5 months.

Blood or wound/bone aspirate cultures were positive in 9/12 of cases. The most common isolate was from the Staphylococcus group. Erythrocyte sedimentation rate (ESR) was elevated in 11 of 12 patients at the time of admission. Meanwhile, white blood count (WBC) and C-reactive protein (CRP) was elevated in only 3/12 of cases. The mean ESR, CRP, and WBC in patients were 62.33 mm/hour, 3.96 mg/dL, and 9.2 x10^3/μL, respectively.

We evaluated clinical outcomes of the patients using the HAQ-DI. Two patients had moderate to severe disability (C-HAQ-DI score 1-2). The other patients experienced mild to moderate disability (C-HAQ-D score 0 to 1).

All patients were treated surgically. Most patients underwent debridement and sequestrectomy. Surgical debridement was done after a minimum of six weeks antibiotic administration. Surgery was done only if there were signs of acute exacerbation. Two patients had histories of two previous surgeries and one patient had had one previous surgery. The other nine patients had not had surgeries prior to admission to our hospital. Patients' data are presented in **Table 1**.

Discussion

In this case series, we present twelve pediatric patients with chronic osteomyelitis. We found that most of the patients had presented to healthcare worker before admission to our hospital, except one who had gone to a traditional bonesetter. However, the average delay of presentation to an orthopedic surgeon was 10.5 months. Such delays are common in developing countries where people often seek initial treatment from the traditional bonesetter.[9] In Indonesia, treatment by bonesetters is associated with complications.[10] In another study, patients delay visiting the doctor due to lack of transportation, low educational status, occupation, or other social reasons.[11] Delays pose an unfavorable prognosis for patients, as a delay of as little as five days was identified to be a risk factor for worse prognosis.[12]

The mean age at presentation in our study was eight years, similar to studies in the Philippines and Cambodia.[4,13] The majority of our patients were male (7/12), in accordance with another study.[14] The predominant anatomical locations of infection were the femur (7/12) and tibia (3/12), also similar to other studies.[13,15] The metaphysis of the long bones was most often involved because of its rich vascular supply.

One unusual case involved the calcaneus. The calcaneus has an apophysis which is equivalent to a long bone's metaphysis, which may explain the development of infection at the site.[16] The diagnosis itself is challenging, as heel pain is often diagnosed as Sever's disease in children, usually leading to delayed diagnosis.[17] Such delays in diagnosis of osteomyelitis of the calcaneus has also been associated with worse prognosis.[18] Indeed, our nine-year-old male patient underwent an amputation below the knee.

Common morbidities in our patients were swelling (3/12), pain (3/12), limping (2/12), and stiffness (2/12). Other studies on acute osteomyelitis noted that in addition to swelling, pain, limitation of motion, and redness, fever was also common.[19] A review stated that children with fever of unknown origin (FUO) should be evaluated for acute osteomyelitis, especially during its peak incidence in prepubertal male children.[20]

Erythrocyte sedimentation rate (ESR) was elevated in 11 of 12 patients. However, CRP was elevated in only 3/12 of cases. The CRP is more useful in monitoring acute cases and its decline is associated with better outcomes.[20] Both ESR and CRP are not specific enough to diagnose chronic osteomyelitis.1 Procalcitonin is a sensitive and specific marker to diagnose acute osteomyelitis. However, to increase the sensitivity and specificity, the cut-off point should be 0.4 ng/mL, not the usual 0.5 ng/mL.[21]

The majority of patients usually presents with late stage of the disease, due to inadequate treatment.[22] Misdiagnosis and undertreatment are still common even though the patients sought advice from health care providers early in the course of disease. Likewise, a previous study found that delayed diagnosis of osteomyelitis resulted in greater disability, as well as lower rates of positive culture and biopsy findings.[23]

Table 1. Patients' demographic factors and clinical features

Patient	Age, years	Sex	Delay time, months	WBC/ESR/CRP	Microbial culture	Morbidity	HAQ-DI score	Number of surgeries	Treatment	First examiner	Location
1	11	Male	3	6/100/0.8	Sterile	Persistent mass	0.45	1	Debridement and sequestrectomy	Nurse	Tibia
2	13	Female	12	5.4/36/0.1	*Staphylococcus haemolyticus*	Stiffness	0.55	1	Debridement and subtalar arthrodesis	GP	Tibia
3	8	Female	12	9.6/90/1.5	Staphylococcus aureus	Persistent mass	1	1	Debridement and curettage	Nurse	Femur
4	9	Male	1.5	17.7/130/5.3	*E. coli*	Amputated	1	3	Debridement and BKA	Ped	Calcaneus
5	6	Male	12	6/13/0.1	*Staphylococcus aureus*	Limping	0.35	1	Debridement	GP	Femur
6	6	Female	18	9/40/0.2	*Staphylococcus aureus*	Swelling	0.32	3	Debridement	GP	Tibia
7	10	Male	12	12.1/80/0.7	*Staphylococcus aureus*	Pain	0.25	1	Debridement, decortication, and sequestrectomy	GP	Femur
8	2	Male	3	12.9/120/3.7	Sterile	Swelling	0.2	1	Debridement and sequestrectomy	Bonesetter	Femur
9	12	Male	12	9/28/0.4	*Mycobacterium tuberculosis*	Limping	0.3	1	Debridement, sequestrectomy and curettage	GP	Femur
10	11	Male	3	6.3/35/0.2	*Staphylococcus aureus*	Bowing	0.2	1	Debridement and sequestrectomy	GP	Humerus
11	6	Female	36	9.3/41/0.2	Sterile	Pathological flexion deformity fracture	0.4	1	Debridement and sequestrectomy	GP	Femur
12	2	Female	1.5	7.5/35/0.8	*Pseudomonas aeruginosa*	Pain	0.4	2	Debridement and curettage	GP	Femur

WBC=white blood cell count (x103/μL); ESR=erythrocyte sedimentation rate (mm/hr); CRP=C-reactive protein (mg/dL); Ped=pediatrician; GP=general practitioner; BKA=below knee amputation

In our cases, routine follow-up was done using plain radiography. Abnormal radiologic findings were found only in one of five patients during the first 7-10 days of the disease. Bone marrow edema, which is the earliest pathological feature, is not visible on plain films. The other radiologic findings of acute osteomyelitis include deep soft tissue swelling, periosteal reaction, and well-circumscribed bone lucency (signifying abscess formation).[24,25] A study reported that osteolytic lesions due to bone destruction were not usually visible until 2-3 weeks after the initial symptoms, but 30-50% of the bone was already destroyed at that point. Bone scintigraphy is a sensitive alternative, but it is not very specific in diagnosing osteomyelitis.[26] MRI is the best radiologic, diagnostic tool for osteomyelitis as it is able to visualize the bone marrow edema. However, CT-scan can also be used when MRI is not possible or contraindicated, as it can be used to visualize the sequestrum.[27] Blood culture was positive only in half of cases. In chronic cases, the results are often polymicrobial. If the blood culture is negative, further investigation by bone biopsy may be done.[2]

We mainly treated our patients with surgical debridement, the standard treatment for chronic osteomyelitis in children. Implementation of reconstructive and stabilization procedures are important to ensure adequate blood supply for new bone formation. Recent advancements also incorporate use of antibiotic-laden cement and vacuum assisted closure (VAC) system.[28,29] However, despite the debridement procedures, one patient still retained marked deformity as depicted in **Figure 2a-c**.

The diagnosis and treatment of pediatric musculoskeletal infection remain a challenge. Combinations of new diagnostic methods, antibiotic resistance, as well as new types of medications and immunizations have changed the epidemiologic pattern of this disease in recent years.[30]

In conclusion, diagnosis of acute hematogenous osteomyelitis in children is quite difficult, given the lack of specific laboratory and radiographic tests. Even joint aspiration is not 100% reliable in diagnosing infection of bones and joints.[31] Clinicians should learn more about acute hematogenous osteomyelitis and be more aware in treating patients with complaints relevant to osteomyelitis. The possibility of musculoskeletal infection must always be considered in clinical practice. In children with FUO, this disease must be suspected in order to prevent late diagnosis and treatment, which may lead to progression to chronic osteomyelitis. Delayed diagnosis and presentation to an orthopedic surgeon is also still a problem, as reflected by our cases. Close multidisciplinary approaches should be done to solve this problem.[32] Hopefully, early diagnosis and treatment will prevent morbidity and save health care costs considerably.

To our knowledge, this is the first series describing cases of pediatric chronic osteomyelitis. However, this study has several limitations. Other aspects of the patient outcomes should have been explored. Moreover, this study needs to be followed up with a larger sample size and multicenter involvement to provide a better picture of chronic osteomyelitis and its outcomes in the pediatric population of Indonesia.

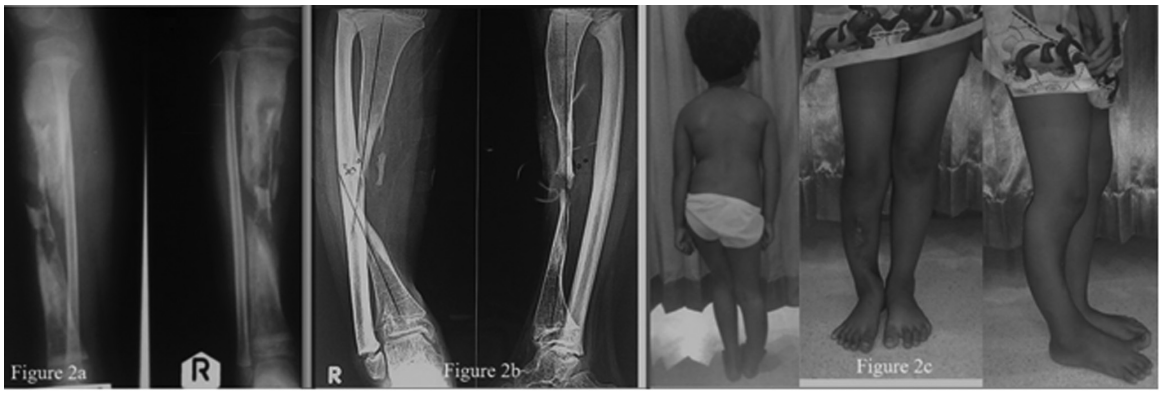

Figure 2. Radiograph of the right tibia on the same patient as in Figure 1. Figure 2(b) shows radiograph two years after two debridement procedures, sequestrectomy and guttering. Figure 2(c) shows the clinical picture of the patient, with scarring and crook deformity on the right leg.

Conflict of Interest

None declared.

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Original Article

Pharmacological treatment strategies for neonates with patent ductus arteriosus: a systematic review

Oliver Emmanuel Yausep[1], Adhi Teguh Perma Iskandar[2]

Abstract

Background A hospital-based cancer registry can be used as Background Patent ductus arteriosus (PDA) has a variety of treatment options, ranging from pharmacologic, with nonsteroidal anti-inflammatory drugs (NSAIDs) as first line therapy, to surgical ligation. However, treatment with NSAIDs is associated with severe side effects as well as many contraindications. Paracetamol is a non-classic NSAID with the prospect of fewer side effects compared to other NSAID counterparts.

Objectives To compare the efficacy and safety of paracetamol to ibuprofen or indomethacin for neonates with PDA by systematic review of the literature.

Methods Our literature search was conducted on four databases: PubMed, Scopus, Ovid, and The Cochrane Library, to find studies that compared paracetamol to ibuprofen or indomethacin in neonates with PDA. Articles were selected based on pre-set eligibility criteria. Outcomes extracted from each study included PDA closure rates as well as adverse events rates.

Results Seven randomized controlled trials (RCTs) were included in this study. Five compared paracetamol to ibuprofen and one used indomethacin as a control. The studies were of good quality, with several variations in methodology. All trials reported similar closure rates of paracetamol compared to ibuprofen or indomethacin. Three studies reported similar rates of adverse events, whereas another three reported safety profiles that favoured paracetamol over ibuprofen.

Conclusion Paracetamol has similar efficacy to ibuprofen and indomethacin with regards to rate of PDA closure following a course of treatment. Paracetamol is also reportedly relatively safe in terms of adverse events rates experienced by patients. **[Paediatr Indones. 2019;59:229-37 ; doi: http://dx.doi.org/10.14238/pi59.5.2019.229-36**].

Keywords: *patent ductus arteriosus; congenital heart disease; NSAID; paracetamol; ibuprofen; indomethacin; systematic review*

Patent ductus arteriosus (PDA) is one of the most common congenital heart diseases and refers to the failure of the ductus arteriosus (DA) to close within 72 hours of birth.[1] During gestation, low fetal blood oxygen, with circulating prostaglandins from the metabolism of arachidonic acid by cyclooxygenase (COX), maintains the patency of the DA.[1] Upon birth, the decreased sensitivity of the DA to prostaglandins causes it to constrict, leading to hypoxia, subsequent remodelling of the duct, and eventually permanent closure.[1] For full term infants, PDA closure is typically achieved within the first 72 hours of life.[2] If delayed, the PDA will close in over 95% of healthy infants by 6 months of age.[3]

An open DA causes a left-to-right shunt of blood from the aorta to the pulmonary artery, adding to the load of the pulmonary circulation. This can eventually lead to pulmonary congestion, edema, and respiratory failure.[2] This shunting also steals blood away from the systemic circulation, compromising

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perfusion to organ systems.[2] Consequentially, PDA is associated with numerous adverse events, including prolongation of assisted ventilation, necrotizing enterocolitis, impaired renal function, cerebral palsy, and death.[4] Determinants of PDA closure include sensitivity to oxygen tension, which promotes closure, and prostaglandin E2, which relaxes smooth muscle and maintains patency.[5] Many conditions can modify these determinants, such as low gestational age, which has been associated with decreased sensitivity to oxygen and increased sensitivity to prostaglandin E2. Concurrent infections have also been shown to produce prostaglandins which contribute to late closure.[1,3]

A number of treatment options that have been implemented for PDA involve inhibition of the COX enzyme that produces prostaglandins. These treatments include indomethacin, ibuprofen, and paracetamol.[3,6] Indomethacin is an NSAID that was first introduced to treat PDA due to its potent vasoconstrictor properties. It is also recommended for prophylaxis, but is associated with side effects due to excessive vasoconstriction, such as impaired renal function, white matter damage, and intestinal perforation.[6] Ibuprofen is another NSAID that has milder side effects compared to indomethacin due to its weaker vasoconstrictor capacity. However, both NSAIDs have been implicated in nephrotoxicity due to prostaglandin suppression, which is requisite for neonatal renal adaptation and development.6 In addition, various contraindications for NSAID use, such as hematuria, blood in stool, sepsis, pulmonary hemorrhage, and renal dysfunction, limit the use of these NSAIDs for PDA, leaving physicians with surgical ligation as a last resort.[6]

Recently, paracetamol, a non-classic NSAID, was shown to be safer and associated with fewer side effects compared to the former two medications. One study reported that paracetamol administration was associated with lower urinary PGE2 reduction and incidence of oliguria, indicating less nephrotoxicity.[7] This greater safety profile compared to classical NSAIDs may be attributed to paracetamol's different mechanism of action, which is in the perioxidase region of the COX enzyme.[8] Using an evidence-based case review, we aimed to compare the efficacy and safety of paracetamol to that of ibuprofen and indomethacin in treating PDA.

Methods

In accordance with the aim of this review, we made the following clinical question, "Which drug, among paracetamol, ibuprofen, and indomethacin, is the most effective and safe to induce PDA closure in preterm neonates?" **Table 1** shows the patient, intervention, comparison, and outcome (PICO) framework used in our review.

This systematic review was written according to the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) guidelines.[9] The literature search was performed on the February 15, 2018, using PubMed, The Cochrane Library, Scopus, and Ovid. The keywords used were "patent ductus arteriosus," "PDA," "neonates," "infants," "children," "paracetamol," "ibuprofen," "indomethacin," and "echocardiography." From PubMed, The Cochrane Library, Scopus, and Ovid, we found 219, 13, 45, and 13 articles, respectively (**Table 2**).

Inclusion criteria included studies that compared the effects of paracetamol in one group of PDA patients, to ibuprofen or indomethacin in another group of PDA patients, by quantifying closure after a course of treatment. Studies that combined treatments, performed studies on adult populations or were in any language than other English or Indonesian were excluded. All study types that fit the inclusion criteria were included.

The selected studies were critically analyzed, by consensus of all authors, using the Critical Appraisal for Randomized Controlled Trials checklist from www.

Table 1. PICO

Patient	Intervention	Comparison	Outcome
Neonate with PDA	Paracetamol	Ibuprofen and/or Indomethacin	Primary: percent of PDA closure after courses of treatment as evaluated by echocardiography Secondary: rate of adverse events
Study type: therapy			

Table 2. Literature search strategy

Database (date)	Applied search keywords	Hits
Pubmed (15/02/2019)	((((((((((patent ductus arteriosus[Title/Abstract]) OR pda[Title/Abstract])) AND (neonates[Title/Abstract]) OR neonate[Title/Abstract]) OR infant[Title/Abstract]) OR infants) OR children))) AND ((((Paracetamol[Title/Abstract]) OR Acetaminophen[Title/Abstract]) AND (Ibuprofen[Title/Abstract]) OR Indomethacin[Title/Abstract]))) AND (echocardio*[Title/Abstract])	219
Cochrane (15/02/2019)	patent ductus arteriosus OR PDA in Title Abstract Keyword AND Echocardiography OR echocardiogram in Title Abstract Keyword AND neonate OR neonates OR infant OR infants OR children in Title Abstract Keyword AND Paracetamol OR Acetaminophen in Title Abstract Keyword AND Ibuprofen OR Indomethacin	13
Scopus (15/02/2019)	patent AND ductus AND arteriosus OR PDA) AND TITLE-ABS-KEY (neonate OR neonates OR infants OR children) AND TITLE-ABS-KEY (paracetamol OR acetaminophen) AND TITLE-ABS-KEY (ibuprofen OR indomethacin) AND TITLE-ABS-KEY (echocardiogram OR echocardiography)	45
Ovid (15/02/2019)	patent AND ductus AND arteriosus OR pda) AND TITLE-ABS-KEY (neonate OR neonates OR infants OR children) AND TITLE-ABS-KEY (paracetamol OR acetaminophen) AND TITLE-ABS-KEY (ibuprofen OR indomethacin) AND TITLE-ABS-KEY (echocardiogram OR echocardiography) patent AND ductus AND arteriosus OR pda) AND TITLE-ABS-KEY (neonate OR neonates OR infants OR children) AND TITLE-ABS-KEY (paracetamol OR acetaminophen) AND TITLE-ABS-KEY (ibuprofen OR indomethacin) AND TITLE-ABS-KEY (echocardiogram OR echocardiography)	13

cebm.net which was developed by Oxford University.[10] The data extracted from each paper included study design, patient characteristics (population criteria, disease, and treatment received), PDA closure after first and second courses of treatment as the primary outcome and adverse events between groups as the secondary outcome. We aimed to compare the rate of PDA closure as a function of treatment course from the evaluated treatments, as well as to review the adverse effects of each treatment, in this systematic review.

Results

A total of 290 articles were found from the four databases. After title and abstract screening, 13 papers were selected for full text review. Subsequently, five duplicates were removed. Full text review yielded 8 viable articles, including 1 article describing a trial that had not been completed, hence, it was excluded. A remaining 7 articles were included in this study (**Figure 1**).

Five studies compared oral paracetamol to oral ibuprofen; one study compared enteral paracetamol to intravenous indomethacin; and one study compared intravenous administrations of paracetamol, ibuprofen, and indomethacin. The studies included were all done in preterm neonates with varying criteria for gestational ages and PDA confirmation. All studies reported the percentage of closed ducts following the first or second course of treatment by a second echocardiogram, as a primary outcome. Associations with adverse events were secondary outcomes. A summary of the studies' designs are compiled in **Table 3**.[8,11-16]

Outcomes from all studies were similar in that paracetamol was equally effective in closing PDA as compared to ibuprofen or indomethacin. Adverse effects observed varied from study to study. The trials by Bagheri *et al.*,[8] Balachander *et al.*,[11] Dang *et al.*,[12] and El-Mashad *et al.*[16] associated paracetamol with less adverse reactions compared to ibuprofen and indomethacin, including hyperbilirubinemia, acute kidney injury, renal dysfunction, thrombocytopenia and gastrointestinal bleeding The other studies demonstrated similar rates of adverse reactions between paracetamol and ibuprofen or indomethacin (**Table 3**).[13-15]

To assess the quality of the selected studies, a critical appraisal tool from Oxford CEBM was used.[10] Overall, the studies appeared to have good quality, with slight variations in attempts at blinding and intention-to-treat analyses. Studies were also relatively recent and had similar designs (**Table 4**).

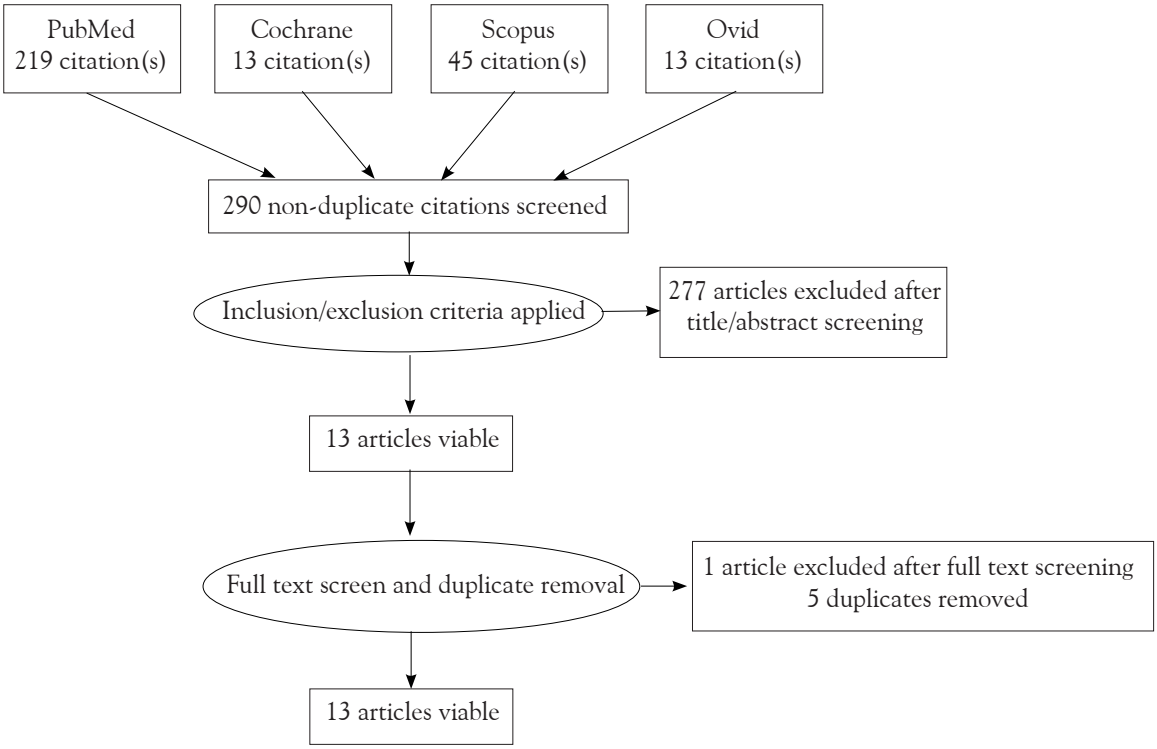

Figure 1. Flowchart of search strategy

Discussion

Congenital heart disease represent one of the most complicated congenital defects in newborns, the most frequent of which is PDA. This congenital heart disease was once associated with poor prognoses, but now has many treatment options ranging from pharmacological therapy with NSAIDs, to surgical ligation. However, the classic NSAIDs used for PDA closure, indomethacin and ibuprofen, are potentially accompanied by detrimental side effects and are also contraindicated in many conditions. Paracetamol, a non-classic NSAID, may serve as an alternative for PDA treatment. In this systematic review, we aimed to compile all relevant evidence to date that directly compared paracetamol efficacy and safety to that of ibuprofen or indomethacin, in achieving PDA closure.

The baseline characteristics of infants in the included studies were preterm infants of gestational age less than 37 weeks, birth weight less than 1,500 g or less than 28 days of life, with ductal-independent PDA, no congenital anomalies, or other clinically concerning conditions. The studies included in this systematic review were all RCTs with a good level of evidence, and published relatively recently (within the past 10 years). Most studies adhered to similar treatment protocols and dosages for drugs used, except for the study by Dash *et al.*[15] which compared intravenous indomethacin with oral paracetamol and El-Mashad *et al.*[16] that compared the three drugs delivered intravenously. A methodological limitation of the studies was the lack of blinding due to the varying dose of one or more drugs. Also, Bagheri *et al.*,[8] did not implement an intention-to-treat analysis, but still retained a drop-out rate of under 20% (14%).

Results from all studies consistently showed that paracetamol was as effective as ibuprofen or indomethacin in closing PDA in preterm infants, with no statistically significant differences between treatment and control groups. This finding held true for the first, second, or even rescue courses of treatment.

Incidence of DA reopening was reported only in studies by Balachander *et al.*,[11] Dang *et al.*[12] and

Table 3. Characteristics of included studies

Author	Patient population	Treatment (intervention and placebo)	Result (% PDA closure)	Conclusion
Bagheri *et al.*[8] (2016)	Preterm neonates (<37 week gestation) with significant PDA	Control: oral ibuprofen 20mg/kg initially, then 10mg/kg after 24 and 48 hours (N=62) Intervention: oral acetaminophen 15mg/kg every 6 hours for 3 days (N=67)	First course: (P= 0.381) • Acetaminophen: 82.1% • Ibuprofen: 75.8% Second course: (P= 0.212) • Acetaminophen: 50% • Ibuprofen: 73.3%	Conclusion: Paracetamol and ibuprofen demonstrated equal efficacy for PDA closure in preterm neonates. Ibuprofen was associated with more adverse effects
Balachander *et al.*[11] (2018)	Preterm neonates with PDA size >1.5mm, with LR shunt after 24 hours of life up to day 28 Control: oral ibuprofen 10mg/kg on day 1 and 5mg/kg 24 hours later (N=55) Intervention: oral paracetamol 15mg/kg every 6 hours for 2 days (N=55) Rescue: an additional course of ibuprofen if the duct failed to close after the first course Stratification: neonates <34 weeks and 34-37 weeks of age		First course: (P=1.00) • Paracetamol: 71.5% • Ibuprofen: 76.4% Rescue course: (P=0.64) • Paracetamol: 18.2% • Ibuprofen: 23.6%	Conclusion: Paracetamol and ibuprofen demonstrated equal efficacy for PDA closure in preterm neonates. Paracetamol was safer and associated with lower risk for acute kidney injury.
Dang *et al.*[12] (2013)	Preterm infants (<34 week gestation), <14 days of age with echocardiographically-confirmed PDA	Control: oral ibuprofen 10mg/kg initially, then 5mg/kg after 24 and 48 hours (N = 80) Intervention: oral paracetamol 15mg/kg every 6 hours for 3 days (N=80)	First course: (P= 0.268) • Paracetamol: 56.3% • Ibuprofen: 47.5% Second course: (P=0.379) • Paracetamol: 25% • Ibuprofen: 31.3%	Conclusion: Paracetamol and ibuprofen demonstrated equal efficacy for PDA closure in preterm neonates. Paracetamol was safer and associated with significantly less hyperbilirubinemia or gastrointestinal bleeding.

Table 3. Characteristics of included studies (continued)

Author	Patient population	Treatment (intervention and placebo)	Result (% PDA closure)	Conclusion
Oncel *et al.*[13] (2014)	Preterm infants (<32 weeks gestation), birthweight <1250g, 48-96 hours of age with echocardiographically- confirmed PDA size >1.5mm		Control: oral ibuprofen 10mg/kg initially, then 5mg/kg after 24 and 48 hours (N=40) Intervention: oral paracetamol 15mg/kg every 6 hours for 3 days (N=40) First course: (P= 0.6) • Paracetamol: 72.5% • Ibuprofen: 77.5% Second course: (P=0.43) • Paracetamol: 27.5% • Ibuprofen: 32.5%	Conclusion: Paracetamol and ibuprofen demonstrated equal efficacy for PDA closure in preterm neonates. Rates of adverse reactions were similar in both groups.
Al-Lawama *et al.*[14] (2017)	Preterm infants (<32 weeks gestation), or birthweight <1500g, with echocardiographically- confirmed, hemodynamically significant PDA	Control: oral ibuprofen 10mg/kg daily for 3 days (N = 9) Intervention: oral paracetamol 10mg/kg every 6 hours for 3 days (N=13)	First course: (P= 0.658) • Paracetamol: 69.2% • Ibuprofen: 77.8% Rescue Course: (P=N/A) • Paracetamol: 23% • Ibuprofen: 11.1%	Conclusion: Paracetamol and ibuprofen demonstrated equal efficacy for PDA closure in preterm neonates. Rates of adverse reactions were similar in both groups
Dash *et al.*[15] (2014)	Preterm infants (<32 weeks gestation), birthweight <1500g, 48-96 hours of age with echocardiographically- confirmed PDA size >1.5mm	Control: intravenous indomethacin 0.2mg/kg daily for 3 days (N = 36) Intervention: enteral paracetamol 15mg/kg every 6 hours for 7 days (N=37)	After treatment: (P= 0.13) • Paracetamol: 100% • Indomethacin: 94.6%	Conclusion: Paracetamol and indomethacin demonstrated equal efficacy for PDA closure in preterm neonates. Neither drug exhibited significantly more adverse reactions, particularly hepatotoxicity.
El-Mashad *et al.*[16] (2016)	Preterm infants (<28 weeks gestation), birthweight <1500g, echocardiographically confirmed PDA	Group 1: paracetamol 15mg/kg IV for 30 mins, followed by 15mg/kg/6hours IV infusion for 3 days (N=100) Group 2: ibuprofen 10mg/kg IV, followed by 5mg/kg/day for 2 days (N=100) Group 3: indomethacin IV 0.2mg/kg for 30 mins for 3 doses at 12-hour intervals (N=100)	First course: (P=0.868) • Paracetamol: 80% • Ibuprofen: 77% • Indomethacin: 81% Second course: (P=0.868) • Paracetamol: 8% • Ibuprofen: 6% • Indomethacin: 6%	Conclusion: Paracetamol, ibuprofen, and indomethacin demonstrated equal efficacy for PDA closure in preterm neonates. Paracetamol was significantly safer with regards to the incidence of gastrointestinal tract bleeding, thrombocytopenia, and renal dysfunction (serum creatinine levels)

N/A: Not available in full text

Table 4. Critical analysis of all studies

Articles	Relevance				Validity							Applicability		
	Domain	Determinant	Outcome	Levels of evidence*	Study design	Number of patients	Randomization	Similarity at baseline	Blinding	Equality outside treatment	Accountability	Applicability to patient	Clinically important outcomes	Benefits > cost?
Bagheri *et al.*[8] (2013)	+	+	+	2	RCT	129	+	+	-	+	-	+	+	+
Balachander *et al.*[13] (2014)	+	+	+	2	RCT	124	+	+	+/-	+	+	+	+	+
Dang *et al.*[12] (2013)	+	+	+	2	RCT	160	+	+	-	+	+	+	+	+
Oncel *et al.*[15] (2014)	+	+	+	2	RCT	80	+	+	+/-	+	+	+	+	+
Al-Lawama *et al.*[14] (2013)	+	+	+	2	RCT	22	+	+	-	+	+	+	+	+
Kumar *et al.*[12] (2014)	+	+	+	2	RCT	73	+	+	-	+	+	+	+	+
El-Mashad *et al.*[16] (2016)	+	+	+	2	P-cohort	300	+	+	+/-	+	+	+	+	+

RCT: Randomized controlled trial; + Clearly stated in the article; - Not done in the article; ? Not clearly stated; * Levels of evidence based on *The Oxford Centre of Evidence Based Medicine 2011*[10] +/- blinding, and no blinding, were done in echocardiography, and drug administration, respectively.

Oncel *et al.*[13] These reopening rates varied between 7-24%, and were similar in both the paracetamol and ibuprofen groups. Of the reopened ducts, a reclosure rate of >60% was observed in all groups that continued treatment, as reported by Dang *et al.*[12] and Oncel *et al.*[13] indicating the possible benefits of extending treatment duration. Dash *et al.*[15] performed the second echocardiographic imaging a full 7 days after administering paracetamol, unlike the other studies, indicating a possibility that spontaneous PDA closure could have taken place prior to that, or may have been due to the additional paracetamol administration. This study was noteworthy in that it was the only study that achieved a 100% closure rate, further supporting the notion that a longer-term paracetamol regimen may be more beneficial for preterm neonates with PDA.

In the studies by Oncel *et al.*,[13] Al-Lawama *et al.*,[14] and Dash *et al.*,[15] paracetamol was associated with a similar rate of adverse reactions as ibuprofen and indomethacin. In contrast, Bagheri *et al.*,[8] Balachander *et al.*,[11] Dang *et al.*,[12] and El-Mashad *et al.*[16] reported the relative safety of paracetamol, in that it was associated with significantly lower rates of various adverse events such as acute kidney injury, thrombocytopenia, hyperbilirubinemia, gastrointestinal bleeding, liver or renal dysfunction, intraventricular haemorrhage, and retinopathy of prematurity. These findings were consistent with previous studies that supported the relative safety of paracetamol as compared to its NSAID counterparts, making paracetamol an even more attractive pharmacologic option to treat neonates with PDA.[10]

In conclusion, given the consistent findings among all seven studies that demonstrated similar efficacy along with the three studies that reported the relative safety of paracetamol compared to ibuprofen and indomethacin, it is safe to conclude that paracetamol is a viable, first-line treatment option for preterm neonates with PDA.

Recommendations

Future studies with similar designs should aim to implement blinding with the use of an opaque covering for syringes, to blind the clinicians involved in caregiving, but that are pre-prepared by unblinded clinicians.[17] Studies should also aim to implement intention-to-treat analyses so as to maintain similar baseline characteristics of the study population. In accordance with the findings of Dash *et al.*,[15] we recommend that the treatment regimen with paracetamol be extended. This extension may potentially reduce the incidence of re-openings or

even preempt them, should they occur during the extended course of treatment.

Conflicts of interest

None declared.

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Original Article

Outcome predictors in patients with juvenile idiopathic arthritis receiving intra-articular corticosteroid therapy

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Abstract

Background Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease in children. It can continue into adulthood and cause severe joint damage, resulting in disability and decreased quality of life.

Objective To determine the predictors of clinical outcomes in JIA patients receiving intra-articular corticosteroid injections (IACS).

Methods We conducted a retrospective cohort study of children with JIA receiving IACS therapy in Dr. Sardjito General Hospital from 1 January 2012 to 31 December 2017 by reviewing data from medical records. The dependent variables were disabilities and early remission time. Independent variables included age at diagnosis, JIA subtype, duration of disease at first diagnosis, timing of IACS, exposure to oral systemic therapy, as well as anti-nuclear antibodies (ANA), rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) test results. External variables were gender and nutritional status.

Results Of 36 patients who received intra-articular corticosteroid injections, 28 (77.8%) experienced remission, and 16 (50%) experienced disabilities. Female subjects (OR 5.296; 95%CI 1.143 to 24.548; P=0.027) and subjects with ESR >26 mm/h (OR 2; 95%CI 1.259 to 3.170; P=0.043) were more likely to have disabilities. Use of oral corticosteroids for ≤3 months and IACS treatment ≤ 3 months after diagnosis were predictors of early remission time (OR 6.897; 95%CI 1.869 to 25 and OR 3.290; 95%CI 1.195 to 9.091, respectively). However, only oral corticosteroid had a significant correlation in multivariate analysis.

Conclusion Female gender and ESR > 26 mm/h predict disabilities in JIA patients receiving IACS. Duration of oral corticosteroid ≤3 months and early IACS within 3 months of diagnosis correlate to earlier remission time. Shorter duration of oral corticosteroid is the only significant predictor for earlier remission time in JIA patients receiving IACS therapy. **[Paediatr Indones. 2019;59:237-43 ; doi: http://dx.doi.org/10.14238/pi59.5.2019.237-43**].

Keywords: *juvenile idiopathic arthritis; intraarticular injection; remission; disability; early remission time*

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease in children, causing severe joint damage which results in disability and decreased quality of life.[1] The aims of therapy are to resolve inflammation, prevent further cartilage damage, and maintain joint function. The mainstay of JIA therapy includes a combination of non-steroidal anti-inflammatory drugs, disease-modifying antirheumatic drugs (DMARDs), biological agents, systemic corticosteroids, intra-articular corticosteroid injections (IACS), and physiotherapy.[2,3] The IACS can significantly reduce pain, improve joint function, as well as promote the repair of bone deformity and bone growth.[3-5]

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Of the limited studies on JIA in Indonesia, most are descriptive, and to our knowledge, none have examined JIA patients who received IACS.[6,7] Identifying predictors of outcomes in JIA patients who received IACS can help in therapy selection for patients and education for both patients and families.[1] We aimed to identify possible outcome predictors in JIA patients receiving intra-articular corticosteroid injections (IACS).

Methods

This retrospective cohort study retrieved data from medical records in Dr. Sardjito General Hospital, Yogyakarta. Subjects were children with JIA aged 1-18 years, diagnosed according to *International League of Associations for Rheumatology* (ILAR) criteria (arthritis that occurs under the age of 16 years with duration of illness 6 weeks or more where other causes of arthritis were excluded)[8] and had received intra-articular corticosteroid injections, as outpatients or inpatients between January 2012 and December 2017. Sampling was not performed as all patients who met the inclusion criteria were enrolled as subjects.

Dependent variables were time to remission and disability. Time to remission was calculated since the time of diagnosis to remission and criteria for disability was adapted from *American College of Rheumatology* revised criteria for classification of functional status in rheumatoid arthritis, which subjects who entered class functional 2-4 were defined to have disabilities. Independent variables were age at diagnosis, JIA subtype, duration of disease at the time of diagnosis, timing of IACS, exposure to oral systemic therapy, anti-nuclear antibodies (ANA), rheumatoid factor (RF), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) test results. External variables were gender and nutritional status. Early corticosteroid injection was defined as corticosteroid injections performed ≤ 3 months after JIA diagnosis.[4]

Data were analyzed using *SPSS Statistics version 22.0* software. Normality of data distribution was determined by the Kolmogorov-Smirnov test. Independent variables were analyzed by Chi-square or Fisher's exact tests for categorical variables, followed by multivariate analysis as appropriate. Predictors of early remission time were analyzed using Kaplan-Meier and Cox-regression analyses. The Medical and Health Research Ethics Committee of the Universitas Gadjah Mada and Dr. Sardjito General Hospital approved this study.

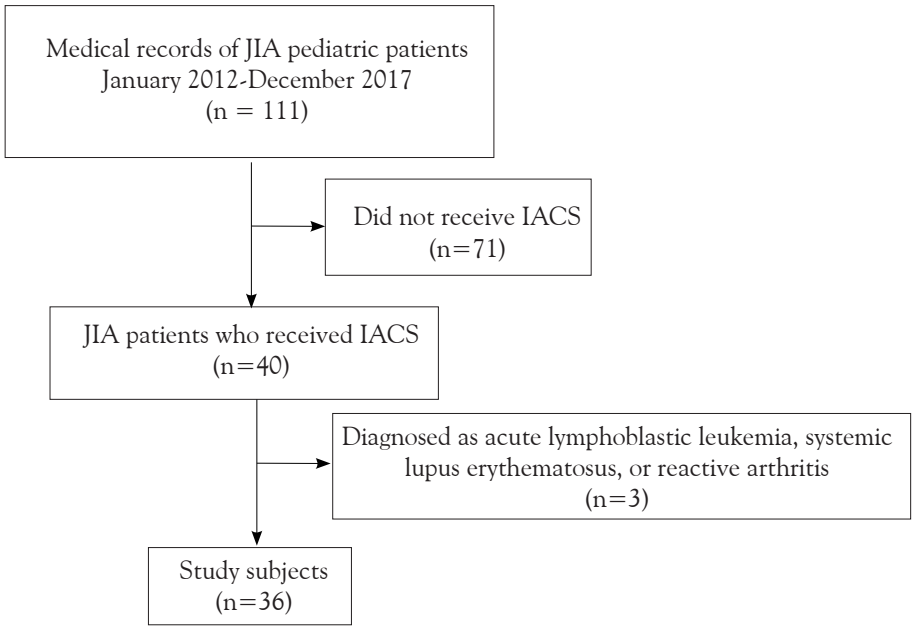

Figure 1. Study subject flow chart

Results

There were 111 JIA patients in RSUP Dr Sardjito from 2012 to 2017, of whom 40 received IACS. We excluded 4 patients due to incomplete medical records or misdiagnosis (**Figure 1**).

Our study included 36 subjects, consisting of 22 girls (61.1%) and 14 boys (38.9%) with a ratio of 1.6:1. Twenty-eight (28) children (77.8%) were in the age group of >6 years. Underweight nutritional status, defined as BMI below 18.5, was found in 22 subjects (61.1%) at the time of enrollment. The most common subtype of JIA was oligoarticular (19 subjects; 52.8%) (**Table 1**).

Table 1. Subjects' characteristics

Characteristics	(N = 36)
Gender	
Female	22
Male	14
Age	
> 6 years	28
≤ 6 years	8
BMI status	
Underweight	22
Normal	13
Overweight	1
Obese	0
Diagnosis	
Oligoarticular	19
Polyarticular RF-	14
Systemic	2
Polyarticular RF+	1

BMI=body mass index; RF+=positive rheumatoid factor; RF-=negative rheumatoid factor

Remission was achieved by 28/36 subjects, with time to achieve remission ranging from 3-37 months after diagnosis (median of 4 months). There were 16 subjects with disabilities (50%). The most common symptoms were joint pain (36/36) and joint swelling (27/36). The ANA was positive in 4 of 21 subjects examined, while RF was positive in 3 of 30 subjects examined. Thirty of 36 subjects received IACS ≤ 3 months after diagnosis (**Table 2**).

Bivariate analysis of predictors for disability is presented in **Table 3**. Female gender (OR 5.296; 95%CI 1.143 to 24.548; P=0.027) and ESR >26 mm/hour (OR 2; 95%CI 1.259 to 3.170; P=0.043) had significantly greater likelihoods for disability in bivariate analysis. However, none of those predictors were significant in multivariate analysis.

Table 2. Clinical and laboratory profiles before IACS therapy

Profiles	(N= 36)
Duration of illness	
≤ 2 months	7
> 2 months	29
Symptoms	
Joint pain	36
Yes	0
No	
Joint swelling	27
Yes	9
No	
Limp	14
Yes	22
No	
Fever	12
Yes	24
No	
Morning stiffness	5
Yes	31
No	
Anti nuclear antibody	
Negative	17
Positive	4
No data	15
Rheumatoid factor	
Negative	27
Positive	3
No data	6
Erythrocyte sedimentation rate	
≤ 26 mm/hour	6
> 26 mm/hour	17
No data	13
C-reactive protein	
≤ 10 mg/dL	11
> 10 mg/dL	11
No data	14
Duration of methotrexate	
≤ 3 months	14
> 3 months	11
Not available	11
Duration of oral corticosteroid	
≤ 3 months	12
> 3 months	12
Not available	12
Timing of IACS after diagnosis	
≤ 3 months	30
> 3 months	6
Disability	
Yes	16
No	20

Table 3. Bivariate and multivariate analysis of predictors for disability

Predictors	Disability (n= 16)	No disability (n= 20)	Bivariate analysis			Multivariate analysis		
			OR	95%CI	P value	OR	95%CI	P value
Gender								
Female	13	9	5.296	1.143 to 24.548	0.027	0.221	0.016 to 2.971	0.255
Male	3	11						
Age								
≤ 6 years	4	4	1.333	0.276 to 6.442	1.000			
> 6 years	12	16						
BMI								
Abnormal	10	12	1.111	0.288 to 4.290	0.878			
Normal	6	8						
Diagnosis								
Oligoarticular	9	10	0.385	0.096 to 1.536	0.171			
Polyarticular RF+	1	1						
Polyarticular RF-	4	9						
Systemic	2	0						
Duration of illness								
> 2 months	12	13	1.615	0.376 to 6.940	0.718			
≤ 2 months	4	7						
Anti nuclear antibody								
Positive	3	1	9.750	0.780 to 121.839	0.490			
Negative	4	13						
Rheumatoid factor								
Negative	12	14	1.714	0.138 to 21.333	1.000			
Positive	1	2						
Erythrocyte sedimentation rate								
> 26 mm/hour	9	9	2.000	1.259 to 3.170	0.043	0.000	0.000 to ~	0.999
≤ 26 mm/hour	0	5						
C-reactive protein								
> 10 mg/dL	5	6	2.222	0.375 to 13.180	0.659			
≤ 10 mg/dL	3	8						
Duration of methotrexate								
≤ 3 months	6	8	0.900	0.183 to 4.415	1.000			
> 3 months	5	6						
Duration of oral corticosteroid								
≤ 3 months	7	5	1.960	0.387 to 9.934	0.414			
> 3 months	5	7						
Timing of IACS after diagnosis								
≤ 3 months	17	14	4.857	0.486 to 48.574	0.338			
> 3 months	1	4						

OR=odds ratio; CI=confidence of interval

Predictors for early remission time were assessed by Cox regression analysis. Shorter duration of oral corticosteroid use and IACS < 3 months after diagnosis (**Table 4**) had greater likelihoods to achieve earlier remission in each time period (HR 6.897; 95%CI 1.869 to 25 and HR 3.290; 95%CI 1.195 to 9.091, respectively). However, only the duration of oral corticosteroid therapy was statistically significant in the multivariate analysis (HR 5.381; 95%CI 1.359 to 21.307 ; P=0.017).

Discussion

There are several indications for intraarticular corticosteroid injection in JIA. This procedure is used as initial therapy for the oligoarticular subtype to relieve synovitis, and/or as additional therapy if the patient does not respond to NSAIDs. The IACS also reduces the need for long-term oral systemic therapy, reduces joint complications including joint contractures and leg length discrepancy, speeds up the

Table 4. Bivariate and multivariate Cox regression analysis for predictors of early remission time

Predictors	Median remission onset, months	Bivariate analysis			Multivariate analysis		
		HR	95%CI	P value	HR	95%CI	P value
Gender							
Female	3.0	1.577	0.710 to 3.401	0.269			
Male	4.0						
Age							
≤ 6 years	3.0	1.582	0.682 to 3.663	0.285			
> 6 years	4.0						
BMI							
Normal	3.0	1.469	0.615 to 3.507	0.386			
Abnormal	3.0						
Diagnosis							
Oligoarticular	3.0	0.634	0.281 to 1.431	0.272			
Polyarticular RF+	1.0						
Polyarticular RF-	4.0						
Duration of illness							
> 2 months	3.0	0.655	0.220 to 1.954	0.448			
≤ 2 months	4.0						
Anti nuclear antibody							
Positive	4.0	1.032	0.292 to 3.648	0.961			
Negative	3.0						
Rheumatic factor							
Positive	1.0	1.624	0.471 to 5.604	0.443			
Negative	3.0						
Erythrocyte sedimentation rate							
> 26 mm/hour	3.0	1.430	0.511 to 4.005	0.496			
≤ 26 mm/hour	4.0						
C reactive protein							
≤ 10 mg/dL	3.0	1.117	0.424 to 2.950	0.822			
> 10 mg/dL	6.0						
Duration of methotrexate							
≤ 3 months	1.0	2.293	0.879 to 5.988	0.090			
> 3 months	8.0						
Duration of oral corticosteroid							
≤ 3 months	1.0	6.897	1.869 to 25	0.004	5.381	1.359 to 21.307	0.017
> 3 months	8.0						
Timing of IACS after diagnosis							
≤ 3 months	3.0	3.290	1.195 to 9.091	0.021	1.781	0.498 to 6.366	0.375
> 3 months	15.0						

HR=hazard ratios

rehabilitation process, improves gait, and reduces pain. Nowadays, intraarticular injection has been applied to the polyarticular subtype as bridging therapy until disease-modifying antirheumatic drugs (DMARDS) therapy starts working effectively, helping to resolve joint deformity and reducing pain.[9]

In our study, females predominated with a female: male ratio of 1.6:1, similar to other studies which reported a predominance of females with JIA (ratios of 1:3 to 1:5, respectively).[6,10] Females might have greater risk for developing autoimmune diseases because of the two copies of the X chromosome and estrogen which plays a role as a promoting factor of the autoimmune processes.[11] Most of our subjects had BMI values lower than normal (61.1%). To our knowledge, there have been no studies that described the nutritional status of JIA patients. Stavropoulos-Kalinoglou *et al.*[12] in 2009 examined the relationship

between nutritional status and disease activity in adult patients with rheumatoid arthritis, and found that subjects with abnormal BMI (thin and obese) had higher CRP values and more severe disabilities.

Most subjects had complaints for more than 2 months (80.6%) before being diagnosed (mean duration of 6 months). Anderson *et al.*[13] noted that the duration of illness has a strong relationship with response to therapy. Patients with longer duration of illness had worse responses to corticosteroid injections. This finding might be caused by the progression of a biological process in the joints over time which causes a reduction in response to therapy.

A diagnosis of arthritis is clinically established by any signs of swelling in the joints or the presence of two of the following joint symptoms: limited joint movement, joint pain, and increased temperature in the joint area. Some studies stated that joint pain was the most common complaint in JIA.[6,7,14,15] This finding was in agreement with our results, as joint pain was the most common complaint (100%), followed by joint swelling (75%).

Increased ANA levels in JIA indicate the role of the humoral immune system in the pathogenesis of JIA.[15] Among the 21 subjects examined, ANA was positive in 4 subjects. Also, RF was positive in 3 of the 30 subjects examined (10%); the RF are often reported in adults with rheumatoid arthritis and usually indicate progressive disease activity, whereas in JIA, RF is only found in 10% of cases, in accordance with our findings.[7]

Examination of ESR and CRP are useful for identifying disease activity.[15] The mean values of ESR and CRP in our study were 48 mm/hour and 34 mg/dL, respectively, indicating that almost all patients were in the acute phase of the disease or the inflammatory process was still ongoing in the patient. Increased ESR accompanied by clinical symptoms are indicative of the ongoing inflammatory process in the joints.[15,16] In our study, ESR >26 mm/hour might be a predictor for disability according to bivariate analysis. This was consistent with a previous study which stated that ESR > 26 mm/hour correlated with longer onset of remission and more severe disease.[17]

Significant predictor for early remission time was duration of oral corticosteroid use. Oral corticosteroids are commonly used in systemic subtypes of JIA. In addition, they are given to JIA polyarticular patients as bridging therapy, or if there is no response to standard therapy. In the systemic JIA protocol used at Dr. Sardjito General Hospital, oral corticosteroids given for 11 weeks are gradually reduced once there is clinical improvement. Our results were in agreement with that of Singh-Grewal *et al.*[17] who showed that patients who no longer needed oral corticosteroid therapy at less than 3 months after diagnosis had earlier remission time (RR 1.95; 95%CI 1.14 to 3.54; P=0.014).

One of the ultimate goals in JIA management is to alter the course of the disease with early therapy. Not only would patients benefit in the short term from faster disease control, but it could translate into longer term benefit by decreasing the occurrence of damage. Early disease control might also impact on the immunological behavior of JIA and alter the long term disease course, a concept called the "window of opportunity".[11,18] Several previous studies focused on the effect of aggressive early systemic therapy, but not on the early corticosteroid injection.[19] There was one study examining the association between early corticosteroid injection and the achievement of remission during the first 2 years after study enrollment, but the results were not significant.[4] Even though early IACS injections could not be proven as a predictor in our study due to study limitation, it is still a promising domain for future studies.

One limitation of this study was the retrospective design using medical records, which has a risk of lost/incomplete data, such as laboratory variables, as well as information bias. Objective measures for patient outcomes such as the *Child Health Assessment Questionnaire* (CHAQ) could not be used because of limited data resources. The small number of subjects also might have diminished the effect of several important and significant predictors from previous studies, which were not statistically significant in our study.

In conclusion, the median time to achieve remission in JIA patients is 4 months, while 50% of subjects suffer from disability during follow up. Female gender and ESR > 26 mm/hour have greater likelihood for disability in JIA patients undergoing corticosteroid injections although they are not significant in multivariate analysis. Shorter duration of oral corticosteroid use is a predictor for early remission time. In view of the increasing use of IACS

in pediatric rheumatology, there is a need for further investigation of JIA outcomes with a well-designed, prospective cohort study.

Conflict of interest

None declared.

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Original Article

Cord blood bilirubin, albumin, and bilirubin/albumin ratio for predicting subsequent neonatal hyperbilirubinemia

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Abstract

Background Early discharge of healthy term newborns after delivery has become a common practice, because of medical and social reasons, as well as economic constraints. Thus, the recognition, follow-up, and early treatment of jaundice has become more difficult as a result of early discharge from the hospital. Since the dreaded complication of neonatal hyperbilirubinemia is kernicterus, an investigation which can predict the future onset of neonatal pathological jaundice is needed.

Objective To investigate the predictability of neonatal hyperbilirubinemia by using cord blood bilirubin, albumin and bilirubin/albumin ratio.

Methods This study was conducted on 300 healthy newborns. Umbilical cord blood was used to measure albumin and bilirubin. All infants were regularly followed up to 5th day of life. Neonates were divided into two groups: group A was consisted of neonates who developed jaundice which was in physiological range, while group B was consisted of neonates who developed neonatal hyperbilirubinemia (requiring phototherapy or other modality of treatment). Babies suspected to have bilirubin level which cross physiological limit on any day after birth were subjected to serum bilirubin measurement. Infants whose serum bilirubin level measurement revealed bilirubin levels crossing physiological values were sent to nursery for phototherapy.

Results The incidence of neonatal hyperbilirubinemia was 11%. Statistically significant correlations between cord blood bilirubin, albumin, and bilirubin/albumin ratio to the development of neonatal hyperbilirubinemia were observed. On ROC analysis, cut-off points to predict significant hyperbilirubinemia in newborn were cord blood bilirubin >3 mg/dL (sensitivity 60.61%, specificity 97.63%), albumin <2.4 mg/dL (sensitivity 78.79%, specificity 98.13%), cord blood bilirubin/albumin ratio >0.98 (sensitivity 78.79%, specificity 95.51%).

Conclusion Cord blood total bilirubin, albumin. and bilirubin/albumin ratio are excellent parameters to predict the occurrence of neonatal hyperbilirubinemia. However, cord blood albumin is better compared to cord blood bilirubin and bilirubin/ albumin ratio. **[Paediatr Indones. 2019;59:244-51; doi: http://dx.doi.org/10.14238/pi59.5.2019.244-51**].

Keywords: *cord blood bilirubin; cord blood albumin; cord blood bilirubin albumin ratio; significant hyperbilirubinemia*

Hyperbilirubinemia is the most common clinical condition requiring evaluation and treatment in the newborn and a frequent reason for hospital readmission during the first week of life. Although generally benign, postnatal, transitional phenomenon, a few neonates develop marked potentially hazardous bilirubin levels that can pose a direct threat of serious brain injury.[1] Acute bilirubin encephalopathy (ABE) may ensue and evolve into kernicterus (chronic bilirubin encephalopathy), a permanent, disabling neurologic condition classically characterized by: (1) movement disorders of dystonia and/or choreoathetosis, (2) hearing loss caused by auditory neuropathy spectrum

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disorders, and (3) oculomotor pareses.[1] The genesis of neonatal hyperbilirubinemia reflects the interplay of developmental red blood cell (RBC), hepatic, and gastrointestinal immaturities that result in an imbalance, favoring bilirubin production over hepatic enteric bilirubin clearance.[2]

Almost all newborn infants have serum or plasma total bilirubin (TB) level >1 mg/dL, in contrast to normal adults in whom the normal TB level is <1 mg/dL. Physiological jaundice usually appears on the 2nd to 3rd day, peaks between 3rd to 5th day of life.[3] Jaundice may appear at birth or may appear any time during neonatal period, depending upon the cause.[3] Since we know hyperbilirubinemia has deleterious effects like kernicterus, choreoathetoid cerebral palsy, hearing impairment, and cognitive impairment if not treated in time, thus meticulous screening of newborn is required to detect hyperbilirubinemia. Since bilirubin level typically peaks at 3rd to 5th day of life, healthy newborns should be followed regularly after discharge from hospital. Infants discharged before 72 hours should be seen within 2 days. Infants of lower gestational ages or having other risk factors should be seen earlier.[3] However, regular follow up is practically impossible in underdeveloped and developing nations because of poverty, low education, and cultural practices. Hence, an ability to predict neonatal hyperbilirubinemia becomes very important and life-saving in the context of a underdeveloped and developing country such as India, where costly investigations and regular follow-up are beyond the reach of the vast majority.

Early discharge of healthy, term newborns after delivery has become common practice, because of medical, social reasons and economic constrains.[4] Thus, the recognition, follow up, and early treatment of jaundice has become more difficult. Severe jaundice, and even kernicterus, can occur in some full-term, healthy newborns discharged early with no apparent early findings of haemolysis.[5]

This study aimed to investigate the predictability of neonatal hyperbilirubinemia by using cord blood bilirubin, albumin, and bilirubin/albumin ratio.

Methods

This prospective, hospital-based study was conducted in Department of Paediatrics and Neonatology, *World College of Medical Sciences* (WCMS) Haryana, India, from 17 January 2017 to 30 November 2018. A total of 300 newborn fulfil the predefined inclusion criteria delivered in our hospital were studied. Proper ethical and scientific clearance was taken from concerned hospital department. Proper informed consent was taken from parents after explaining to them the risks and benefits of neonatal jaundice, phototherapy, and blood sampling.

Inclusion criteria were gestational age 35 weeks and above (based on last menstrual period) and the absence of major congenital malformations. The exclusion criteria were presence of significant illness (i.e., sepsis, hypothyroidism), Rh incompatibility, ABO incompatibility, newborns with obvious life-threatening congenital malformation (tracheoesophageal fistula, anorectal malformation, and babies with conjugated hyperbilirubinemia).

All babies delivered in WCMS were examined and detailed antenatal and postnatal histories were taken. Blood was collected from umbilical cord blood from all neonates at birth. Maternal blood samples were simultaneously collected and sent for blood group testing, if it was not known prior. The infant cord blood sample was sent for blood group testing, as well as bilirubin and albumin measurement. All babies were examined every day up to 5th day of life by senior residents for clinical assessment of bilirubin as per Kramer's scale which states, "if on clinical examination we found jaundice in the face and neck only – total serum bilirubin (TSB) >5 mg/dL, jaundice on chest (up to umbilicus and back) – TSB between 5 and 10 mg/dL, jaundice from umbilicus to knees - TSB 10-15 mg/dL, and jaundice in palms and soles – TSB >15 mg/dL and by transcutaneous bilirubinometer for continuous 5 days." Grouping was done on the basis of physiological and neonatal hyperbilirubinemia (hyperbilirubinemia which required phototherapy). Babies whose bilirubin always remain within physiological limits on clinical examination, there bilirubin was checked by serum estimation method on 5th day thus, were included in Group A. Group B babies included those babies who had on both clinical and serum estimation method neonatal hyperbilirubinemia which required phototherapy or other modality of treatment (as per *American Academy of Pediatrician*/AAP nomogram for

hyperbilirubinemia management)[6] on any day from birth up to 5th day of life. Serum bilirubin of group B babies was checked on that very day when on clinical examination crossing of bilirubin level beyond physiological level was suspected.

Serum bilirubin was estimated by micro-bilirubin (Jendrassik & Grof method)[7] using venous blood taken in four microcapillaries and centrifuged at the rate of 10,000 rpm for 5 minutes. Bilirubin measurement was done spectrophotometrically, using beam method (55 nm wavelength) (micro la-300, *Merck*, The Netherlands). Bilimeter calibration was done daily using a labeteral solution.

Data were recorded, properly validated, then checked for errors and then analysed using *Windows SPSS 21* software. Appropriate univariate and bivariate analyses were done with student's T-test for continuous variables (age), and two-tailed Fisher's exact or Chi-square (X^2) tests for categorical variables. Sensitivity, specificity, as well as positive and negative predictive values of different cut-off points of cord blood serum bilirubin were derived. Results with P values <0.05 were considered statistically significant.

This study aimed to investigate the predictability of neonatal hyperbilirubinemia by using cord blood bilirubin, albumin and bilirubin/albumin ratio.

Results

All the 300 newborn were exclusively breastfed, 169 (56.33%) were males. Cord blood bilirubin, serum bilirubin, and cord blood bilirubin/albumin ratio were not signficantly different between males and females (**Table 1**).

Gestational age of two hundred eighteen (72.67%) infants were >37 weeks and 82 (27.67%) 35-37 weeks. Mean cord blood bilirubin, albumin, bilirubin/albumin ratio, and serum bilirubin estimated up to 5th day were not significantly different between the two gestational age categories. Similarly, birth weight and mode of delivery comparison of cord blood bilirubin, albumin, bilirubin/albumin ratio, and serum bilirubin estimated up to the 5th day were also not significantly different (**Table 1**).

A total of 267 (89%) (group A) developed jaundice in physiological range, so went home without treatment. The mean total albumin, bilirubin, and bilirubin/albumin ratio in cord blood were 2.63 (SD 0.26) mg/dL, 2.574 (SD 0.57) mg/dL, and 0.91 (SD 0.14) mg/dL, respectively. Mean serum bilirubin on day 5 of group A was 10.5 (SD 2.1) mg/dL (**Table 2**). No significant differences were revealed within group A subjects when their cord blood parameters (albumin, bilirubin & bilirubin albumin

Table 1. Distribution of subjects by sex, gestational age, birth weight, and mode of delivery

Characteristics	(N=300)	Mean cord blood bilirubin (SD)	Mean cord blood albumin (SD)	Mean cord blood bilirubin/albumin ratio (SD)	Mean bilirubin while monitoring up to 5th day of life (SD)	P value
Sex, n (%)						
Male	169 (56.33)	2.6 (0.8)	2.4 (0.7)	0.96 (0.24)	1.39 (2.4)	0.89[1] 0.90[2]
Female	131 (43.67)	2.5 (0.4)	2.1 (0.8)	0.82 (0.27)	12.9 (1.8)	0.86[3]
Gestational age, n (%)						
35-37 weeks	82 (27.67)	2.4 (1.4)	1.4 (1.1)	0.67 (0.17)	13.2 (2.2)	0.95[1]
>37 weeks	218 (72.67)	2.7 (0.8)	2.9 (0.6)	0.95 (0.24)	13.7 (2.2)	0.68[2] 0.76[3]
Mode of delivery, n (%)						
Vaginal	198 (66)	2.9 (1.3)	2.7 (1.2)	0.86 (0.34	11.9 (2.0)	0.78[1]
Lower segment caesarean section (LSCS)	102 (34)	2.6 (0.8)	2.1 (0.7)	0.79 (0.41)	12.9 (2.4)	0.64[2] 0.78[3]
Birth weight, n (%)						
1.5-2.5 kg	98 (32.67)	2.5 (1.9)	2.4 (1.7)	0.78 (0.56)	14.21 (1.4)	0.999[1]
2.6-3.5 kg	152 (50.67)	2.9 (1.4)	2.8 (1.6)	0.89 (0.7)	12.3 (1.7)	0.84[2]
>3.5 kg	50 (16.67)	2.87 (0.4)	1.9 (0.7)	0.85 (0.75)	12.5 (2.8)	0.57[3]

[1]=P value for cord blood bilirubin association with characteristics
[2]=P value for cord blood albumin association with characteristics
[3]=P value for cord blood bilirubin/albumin ratio association with characteristics

ratio) were compared with bilirubin on 5th day of their life (**Table 2**).

Prevalence of neonatal hyperbilirubinemia (babies who required phototherapy or other treatment modality) in our study was 33 (11%) (group B). Group B mean cord blood albumin, bilirubin, and bilirubin/albumin ratio were 2.28 (SD 0.32) mg/dL, 3.136 (SD 0.33) mg/dL, and 1.4 (SD 0.35), respectively, and mean serum bilirubin when measured up to the 5th day of life was 16.2 (SD 1.6) mg/dL. Statistical analysis revealed significant correlations of all three parameters with subsequent neonatal hyperbilirubinemia (**Table 2**).

All data collected was analysed for cut-off values in receiver-operating characteristic (ROC) curves. For cord blood albumin, area under curve (AUC) was 0.901,cut-off point of <2.4 mg/dL, good statistical significance with sensitivity of 78.79%, specificity 98.13%, positive likelihood ratio 42.07, negative likelihood ratio 0.22, positive predictive value 83.9, and negative predictive value 97.4, for predicting subsequent neonatal hyperbilirubinemia (P<0.001) (**Table 3** and **Figure 1**).

There was a significant correlation between cord blood bilirubin and development of subsequent neonatal hyperbilirubinemia, with AUC 0.766 (P<0.001) for a cut-off point of >3 mg/dL, which had sensitivity of 60.61%, specificity 97.63%, positive likelihood ratio 23.31, negative likelihood ratio 0.4, positive predicative value 74.1, and negative predictive value 95.2 (**Table 4** and **Figure 2**).

Similarly, cord blood bilirubin/albumin ratio has significant correlation with subsequent development of neonatal hyperbilirubinemia with AUC of 0.896 (P<0.001) at cut-off value of 0.98 with sensitivity of 78.79%, specificity of 95.51%, positive likelihood ratio 17.53, negative likelihood ratio of 0.22, positive predicative value of 68.41, and negative predictive value of 97.3(**Table 5** and **Figure 3**).

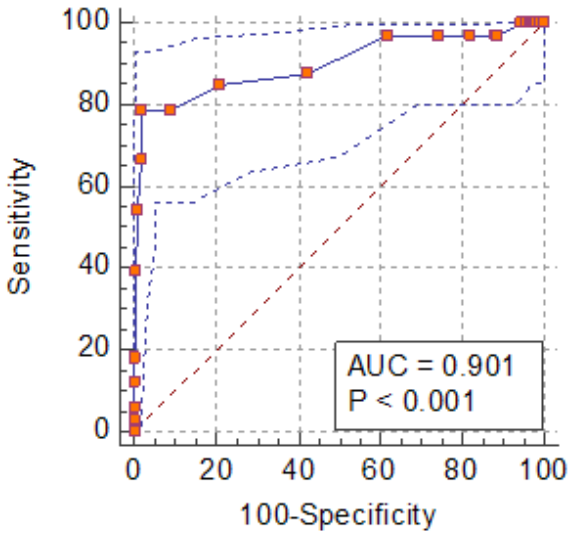

Figure 1. ROC curve for mean cord blood albumin

Table 2. Mean cord blood albumin, bilirubin, bilirubin/albumin ratio, and bilirubin up to 5th day of life

Group	n(%)	Mean cord blood albumin in mg/dL (SD)	Mean cord blood bilirubin in mg/dL (SD)	Cord blood bilirubin/albumin ratio (SD)	Mean bilirubin during monitoring in mg/dL (SD)	P value
A	267 (89)	2.63 (0.26)	2.574 (0.57)	0.91 (0.14)	10.5 (2.1) [on 5th day]	0.097[1] 0.67[2] 0.7[3]
B	33 (11)	2.28 (0.32)	3.136 (0.33)	1.4 (0.35)	16.2 (1.6) [up to 5th day]	0.0001[1] 0.000[2] 0.003[3]

[1]=P value for association of cord blood albumin with future predictability of hyperbilirubinemia
[2]=P value for association of cord blood bilirubin with future predictability of hyperbilirubinemia
[3]=P value for association of cord blood bilirubin/albumin ratio with future predictability of hyperbilirubinemia

Table 3. Area under the curve for cord blood albumin (CBA)

Test result variable: cord blood albumin				Associated criterion					
AUC	Std. error	Asymptomatic sig.	Asymptomatic 95%CI	2.4 mg/dL					
				Sensitivity	Specificity	Positive LR	Negative LR	PPV	NPV
0.901	0.37	0.000	0.828 to 0.975	78.79%	98.13%	42.07	0.22	83.9	97.4

LR=likelihood ratio; PPV=positive predictive value; NPV=negative predictive value

Table 4. Area under the curve for cord blood bilirubin (CBB)

Test result variable: cord blood bilirubin				Associated criterion					
AUC	Std. error	Asymptomatic sig.	Asymptomatic 95%CI	3 mg/dL					
				Sensitivity	Specificity	Positive LR	Negative LR	PPV	NPV
0.766	0.055	0.000	0.658 to 0.874	60.61%	97.63%	23.12	0.40	74.1	95.2

LR=likelihood ratio; PPV=positive predictive value; NPV=negative predictive value

Table 5. Area under the curve for cord blood bilirubin/albumin ratio (BAR)

Test result variable: cord blood bilirubin/albumin ratio				Associated criterion					
AUC	Std. error	Asymptomatic sig.	Asymptomatic 95%CI	0.98					
				Sensitivity	Specificity	Positive LR	Negative LR	PPV	NPV
0.896	0.0392	0.000	0.856 to 0.928	78.79%	95.51%	17.53	0.22	68.4	97.3

LR=likelihood ratio; PPV=positive predictive value; NPV=negative predictive value

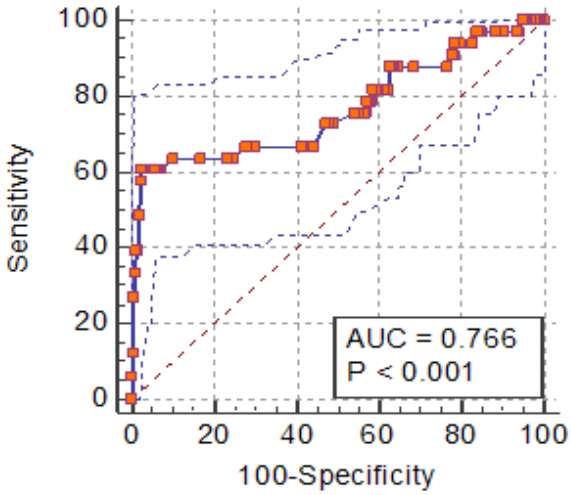

Figure 2. ROC curve for mean cord bilirubin

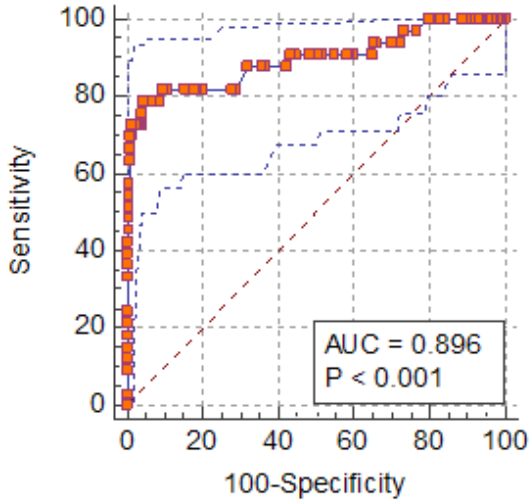

Figure 3. ROC curve for mean cord blood bilirubin/

Table 6. ROC curve statistics of parameters.

Parameters	AUC	SE	95% CI
CBA	0.901	0.0378	0.862 to 0.932
CBB	0.766	0.0559	0.714 to 0.813
BAR	0.896	0.0392	0.856 to 0.928

CBA=cord blood albumin; CBB=cord blood bilirubin; BAR=bilirubin albumin ratio; AUC=area under curve

The AUC values for predictive ability of cord blood albumin, bilirubin, and bilirubin/albumin ratio for subsequent neonatal hyperbilirubinemia were: CBA (0.901) > BAR (0.896) > CBB (0.766) (**Table 6**). Statistical analysis revealed significant differences between CBB-BAR (P=0.003) and CBA-CBB (P=0.0297). However, CBA-BAR AUCs were not significantly different (P=0.9032) (**Table 7** and **Figure 4**).

Discussion

Jaundice is a common entity in newborn which requires attention in the first few days after birth. Most jaundice which develops in newborn is in physiological range, except small fraction which need intervention like phototherapy, exchange transfusion, or other new modalities of treatment. Although about 8-10% are affected, as shown in our study with 11%, timely diagnosis and immediate treatment is essential to prevent the devasting effects of kernicterus, which can lead to mental retardation, choreoathetoid type of cerebral palsy, or hearing defects. These side effects have dramatically decreased in last decade because of public awareness educational programs. But there

Table 7. Pairwise comparison of ROC curves

Group comparison	Difference between areas	Standard error	95%CI	Z statistic	P value
CBA~CBB	0.135	0.0620	0.0133 to 0.257	2.174	0.0297
CBA~BAR	0.00499	0.0411	-0.0755 to 0.0855	0.122	0.9032
CBB~BAR	0.130	0.0360	0.0594 to 0.200	3.612	0.0003

CBA=cord blood albumin; CBB=cord blood bilirubin; BAR=bilirubin albumin ratio

remains a small fraction of newborn who fall prey to the devasting side effects of neonatal hyperbilirubinemia, especially in developing countries, because of poor follow up, limited resources, and most importantly parental emotional attachment. Some parents do not want their child to experience pain, even from a single needle prick. Keeping in view all these factors, the objective of this research was framed to determine the cut-off values for cord blood bilirubin, albumin, and bilirubin/albumin ratio, with hope that such values could be used to predict development of subsequent neonatal hyperbilirubinemia. We also compared these three parameters to determine which one is better.

The prevalence of significant hyperbilirubinemia in our study was 11%. Similarly, Awasthi *et al.*[8] reported 12.80%, Randev *et al.*[9] reported 12.00%, and Dhanwadkar *et al.*[10] reported 11.4%. In our study, there were no significant relationships between neonatal hyperbilirubinemia and cord blood bilirubin, albumin, and bilirubin/albumin ratio, with regards to gender, gestational age, birth weight, or mode of delivery. Similar findings were noted by Awasthi *et al.*[8] and Alpay *et al.*[11]

In our study, mean cord blood albumin of babies who developed neonatal hyperbilirubinemia which required treatment was 2.28 (SD 0.32) mg/dL, similar to the finding of Aiyappa *et al.*[12] On ROC curve analysis, the cord blood albumin cut-off point to predict subsequent neonatal hyperbilirubinemia was <2.4 mg/dL. Pahuja *et al.*[13] noted that the predictive value of cord albumin for development of neonatal hyperbilirubinemia was 75%, which implied a fair predictive value of the criteria, with 61.3% sensitivity and 76.8% specificity, and was in agreement with our study. Thakur P *et al.*[14] found 4% incidence of neonatal hyperbilirubinemia at cord blood albumin level cut-off of <2 mg/dL, with specificity of 98.23%. Also, Mahmoud Alalfy *et al.*[15] noted the highest sensitivity (83.3%) was for cord bilirubin cut-off value 1.8 8mg/dL, with PPV 72.9%, which means that 83.3% of patients can be predicted to have the disease (true positives), but 16.7% of cases with the disease will go undetected (false negatives).

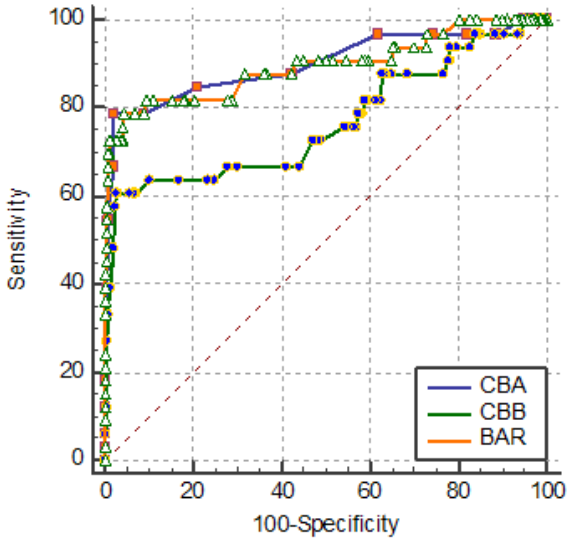

Figure 4. Comparison of ROC curves of cord blood albumin (CBA), cord blood bilirubin (CBB), and bilirubin albumin ratio (BAR)

The mean cord blood bilirubin of newborn who developed neonatal hyperbilirubinemia was 3.136 (SD 0.33) mg/dL, which was same as shown by a previous study that reported a mean value of 3.2 (SD 0.8) mg/dL.[16] The ROC curve analysis cord blood bilirubin cut-off point was > 3.0 mg/dL, with sensitivity 60.61%, specificity 97.63%, positive likelihood ratio 23.31, negative likelihood ratio 0.4, PPV 74.1, and NPV 95.2. Similarly, Taksande *et al.*[17] noted the cut-off value of 2.0 mg/dL, with sensitivity 89.5% and NPV 98.7%. However, at the cut off value of 2.0 mg/dL, 53% sensitivity was observed by Bernaldo and Segre[16] and at 2.5 mg/dL, 71% sensitivity and 96% specificity were noted by Agarwal *et al.*[18] In addition, another previous study noted that cord blood bilirubin level of 3 mg/dL (51.3 micromol/L) was not a useful predictor of neonatal jaundice.[19] Venkatamurthy *et al.*[20] found that at cord blood bilirubin level ≥2.1

mg/dL, sensitivity was 100%, specificity 61.04%, PPV 25%, and NPV 100%.

The ROC curve analysis of the bilirubin/albumin ratio in cord blood revealed that, cut-off point of >0.98 was predictive with good accuracy of development of subsequent neonatal hyperbilirubinemia. Similarly, a previous study reported a cord blood bilirubin/albumin ratio cut-off of 0.82, as obtained by ROC curve, and with 88.9% sensitivity, 85.7% specificity, PPV 94.1%, and NPV 75% for predicting neonatal hyperbilirubinemia in a high-risk group.[15] Ramteke *et al.*[21] derived a 0.89 cut-off point of cord bilirubin/albumin ratio, and suggested that 95.5% of patients will likely develop future neonatal NNH if their ratio is above 0.89.

On comparing the ROC curves of the three parameters, there were significant differences between CBA and CBB, as well as BAR and CBB, which indicated better predictability of CBA and BAR for the development of subsequent jaundice. However, the comparison of BAR and CBA revealed no significant difference in the two parameters, as P value was greater than 0.05. However, keeping in view other statistical figures like AUC, sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, PPV, and NPV, the CBA as the single parameter would be more useful to predict subsequent significant neonatal hyperbilirubinemia.

In conclusion, there is significant correlation between development of neonatal hyperbilirubinemia and cord blood serum bilirubin, albumin, and bilirubin/albumin ratio. Cord blood albumin has the best predictive value, followed by bilirubin/albumin ratio and cord blood bilirubin in predicting development of subsequent neonatal hyperbilirubinemia.

Conflict of Interest

None declared.

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Original Article

Risk factors of neonatal hypoglycemia

Yuliana Yunarto, Gatot Irawan Sarosa

Abstract

Background Hypoglycemia is the most common metabolic issue in newborns and should be treated as soon as possible to prevent complications of neurologic impairment, mental retardation, developmental delay, and cardiovascular disorders.

Objective To assess maternal, fetal, and neonatal factors for identifying infants at risk of developing neonatal hypoglycemia.

Methods This case-control study was conducted in the Perinatal Unit of Dr. Kariadi Hospital, Semarang, Central Java. A total of 123 newborns with blood glucose <47 mg/dL comprised the case group and 123 newborns without hypoglycemia comprised the control group. Characteristics of infants, maternal age, maternal pregnancy-related conditions, as well as fetal and neonatal factors were recorded and analyzed for possible relationships with hypoglycemia.

Results Out of 677 newborns, hypoglycemia was found in 123 (18.2%) infants (59 male, 64 female). In the case group, 58 (47.1%) were preterm, 38 (30.9%) very preterm, and 8 (6.5%) extremely preterm infants. Factors associated with neonatal hypoglycemia were prematurity (OR 6.537; 95%CI 3.543 to 12.063; P <0.001), low birth weight (OR 2.979; 95% CI 1.532 to 5.795; P<0.001), small for gestational age (OR 1.805; 95% CI 1.054 to 3.095; P=0.031), and birth asphyxia (OR 3.386; 95% CI 1.945 to 5.895; P<0.001). In multivariate regression analysis, prematurity and low birth weight remained the significant factors associated with neonatal hypoglycemia.

Conclusion Prematurity and, low birth weight are significant risk factors associated with neonatal hypoglycemia. Routine screening and monitoring of blood glucose is recommended for preterm newborns and infants with low birth weight. [Paediatr Indones. 2019;59:252-6; doi: http://dx.doi.org/10.14238/pi59.5.2019.252-6].

Keywords: *neonatal hypoglicemia; birth asphyxia; low birth weight; small for gestational age; prematurity*

Hypoglycemia is the most common metabolic issue in newborns and should be treated as soon as possible to prevent complications of neurological impairment, mental retardation, developmental delay, and debilitating cardiovascular function.[1] It is important to recognize hypoglycemia in order to initiate prompt treatment and prevent long-term neurologic damage. Hypoglycemia is mostly asymptomatic, but timely and accurate recognition is important to achieve optimal neonatal outcomes.[2] We aimed to assess maternal, fetal, and neonatal factors for identifying infants at risk of developing neonatal hypoglycemia.

Methods

This case-control study was conducted in the Perinatal Unit of Dr. Kariadi Hospital, Semarang, Central Java, Indonesia. Data were obtained from medical records of infants born in 2017. Fullterm was defined as baby born after 37 weeks of pregnancy; meanwhile, preterm was defined as baby born before 37 weeks of pregnancy and divided into very preterm (28-32 weeks) and extremely preterm (less than 28

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weeks). Babies born with birthweight between 2500 - 3999 g were considered as normal birthweight; low birth weight (LBW) was defined as a birth weight of less than 2500 g which further categorized into very low birth weight (<1500 g) and extremely low birth weight (<1000 g). Birth asphyxia was defined as the failure to establish breathing at birth defined based on the 5th min Apgar score of <7. Blood glucose levels documented in medical records were measured from a heel puncture blood sample.

Patients born to diabetic mothers or with multiple congenital anomalies were excluded. A total of 123 newborns with blood glucose <47 mg/dL comprised the case group. The control group was comprised of 123 newborns without hypoglycemia, included by simple randomization sampling. Characteristics of infants, maternal age, maternal pregnancy-related conditions, as well as fetal and neonatal factors were recorded. Data were analyzed using *SPSS 20* for Windows software. Means and proportions of blood glucose levels and basic socio-demographic data, as well as clinical data, were analyzed by Student's T-test and Chi-square test, respectively. Multivariate analysis was performed to identify significant risk factors related to the occurrence of hypoglycemia. Results with P values < 0.05 were considered to be statistically significant.

The Medical and Health Research Ethics Committee of Dr. Kariadi Hospital approved this study.

Results

Characteristics of subjects are shown in **Table 1**. Of 677 newborns hospitalized in year 2017, hypoglycemia was found in 123 (18.2%) infants (59 male, 64 female). Of the case group, a total of 65 (52.8%) were born via caesarean delivery, maternal age were mostly <35 years (79.7%), and multiparous (65%).

Table 2 shows that in the case group, hypoglycemia was found mostly in infants born less than 37 weeks consisted of 58 (47.1%) preterm, 38 (30.9%) very preterm, and 8 (6.5%) extremely preterm infants. A total of 89 (72.3%) were born with low birth weight, 25 (20.3%) were born very low birth weight, and 5 (4%) infants were born with extremely low birth weight. In addition, 49 (39.9%) infants were born small for gestational age (SGA). Factors associated with neonatal hypoglycemia were prematurity (OR 6.537; 95% CI 3.543 to 12.063; P<0.001), birth weight less than 2,500 g (OR 2.979; 95%CI 1.532 to 5.795; P<0.001), SGA (OR 1.805; 95%CI 1.054 to 3.095; P=0.031), and birth asphyxia (OR 3.386; 95%CI 1.945 to 5.895; P<0.001).

Multivariate regression analysis as shown in **Table 3** revealed that prematurity and low birth weight were the significant factors associated with neonatal hypoglycemia. None of the other maternal or fetal conditions were associated with the occurrence of neonatal hypoglycemia in our study.

Table 1. Characteristics of subjects

Characteristics	Hypoglycemic (n = 123)	Normoglycemic (n = 123)	P value
Gender, n (%)			
Male	59 (48)	48 (39)	0.158
Female	64 (52)	75 (61)	
Mode of delivery, n (%)			
Caesarean	65 (52.8)	76 (61.8)	0.157
Vaginal	58 (47.2)	47 (38.2)	
Maternal age, n (%)			
≥35 years	25 (20.3)	26 (21.2)	0.875
<35 years	98 (79.7)	97 (78.8)	
Maternal parity, n (%)			
Primiparous	43 (35)	42 (34.1)	0.89
Multiparous	80 (65)	81 (65.8)	

Table 2. Risk factors for neonatal hypoglycemia

Variables	Hypoglycemic (n = 123)	Normoglycemic (n = 123)	Odds ratio (95% CI)	P value
Birth weight to gestational age, n (%)				
SGA	49 (39.9)	33 (26.8)	1.805 (1.054 to 3.095)	0.031*
AGA	74 (60.1)	90 (73.2)		
Gestational age, n (%)				
Full term	19 (15.4)	64 (52)	6.537 (3.543 to 12.063)	<0.001*
Preterm	58 (47.1)	42 (34.1)		
Very preterm	38 (30.9)	13 (10.6)		
Extremely preterm	8 (6.5)	4 (3.2)		
Birth weight, n (%)				
NBW	4 (3.2)	37 (30.1)	2.979 (1.532 to 5.795)	0.001*
LBW	89 (72.3)	79 (64.2)		
VLBW	25 (20.3)	4 (3.2)		
ELBW	5 (4)	3 (2.4)		
Maternal risk factors, n (%)				
PROM	14 (11.4)	14 (11.4)	1.000 (0.455 to 2.197)	1.000
Preeclampsia/ eclampsia	35 (26)	40 (32.5)	0.825 (0.479 to 1.422)	0.489
Placental abnormality	9 (7.3)	5 (4)	1.863 (0.606 to 5.728)	0.278
Maternal infection	5 (4)	10 (8.1)	0.479 (0.159 to 1.444)	0.191
Fetal risk factors, n (%)				
Fetal distress	8 (6.5)	5 (4)	1.642 (0.522 to 5.167)	0.397
IUGR	31 (25.2)	52 (42.3)	1.637 (0.879 to 3.047)	0.120
Gemelli	13 (10.6)	8 (6.5)	1.699 (0.678 to 4.257)	0.258
Neonatal risk factors, n (%)				
Birth asphyxia	60 (48.8)	27 (22)	3.386 (1.945 to 5.895)	<0.001*
Neonatal jaundice	8 (6.5)	24 (19.5)	0.287 (0.123 to 0.667)	0.004
Infection	12 (9.8)	6 (4.8)	2.108 (0.765 to 5.810)	0.149
RDS	4 (3.2)	2 (1.6)	2.034 (0.366 to 11.313)	0.418

NBW=normal birth weight; LBW=low birth weight; VLBW=very low birth weight; ELBW=extremely low birth weight; PROM=premature rupture of the membrane; HELLP=hemolysis, elevated liver enzyme levels, and low platelet levels; IUGR=intrauterine growth restriction; RDS=respiratory distress syndrome

Table 3. Multivariate logistic regression of risk factors for neonatal hypoglycemia

Variables	Odds ratio (95% CI)	P value
Gestational age	7.943 (3.911 to 16.130)	<0.001*
Birth weight	3.833 (1.690 to 8.692)	0.001*
Mode of delivery	0.752 (0.406 to 1.393)	0.365
Birth weight to gestational age	0.905 (0.357 to 2.294)	0.833
Maternal heart disease	0.545 (0.123 to 2.417)	0.424
Maternal infection	0.685 (0.180 to 2.604)	0.578
IUGR	1.630 (0.554 to 4.792)	0.374
Birth asphyxia	1.828 (0.898 to 3.724)	0.096
Neonatal jaundice	0.618 (0.228 to 1.675)	0.345
Neonatal infection	1.576 (0.482 to 5.157)	0.452

Discussion

Among infants born at <37 weeks, we observed an increased risk of neonatal hypoglycemia. The incidences of hypoglycemia among preterm, very preterm, and extremely preterm newborns in our study were 47.1%, 30.9%, and 6.5%, respectively. The prevalence of hypoglycemia in our study was higher than that of Bromiker *et al.*,[2] however, they found that prematurity as the strongest risk factor for neonatal hypoglycemia. Preterm neonates are at risk of developing hypoglycemia and its associated complications due to their low reserve of glycogen and fat stores, inefficient production of glucose using gluconeogenesis pathways, higher metabolic requirement due to a relatively larger brain size, and inadequate ability to escalate a counter-regulatory response to hypoglycemia.[3]

Birth weight was found to be one of the significant risk factors associated with hypoglycemia in our study. In hypoglycemic infants, 72.3%, 20.3%, and 4% were LBW, VLBW, and ELBW infants, respectively. Similarly, Kumar *et al.*[4] reported a higher percentage LBW babies in their hypoglycemic group (64% LBW *vs.* 14% NBW). They found that LBW infants were susceptible to developing hypoglycemia mainly in first 24 hours of life, with late introduction of breastfeeding being an additional risk. Low birth weight neonates are born with low stores of glycogen and adipose tissues as well as inadequate capacity to produce glucose through the gluconeogenesis pathway or disproportionate peripheral tissue utilization of glucose. Hence, such infants are prone to hypoglycemia.[3]

Hypoglycemia was present in 59.8% of the SGA infants. These findings were consistent with those of Ho *et al.*[5] who reported an incidence of 34.2% in SGA infants. The neonatal brain and vital organs need a steady supply of glucose to meet nutritional demands. In full term and healthy neonates, this is accomplished by hormonal and metabolic adaptive changes. Preterm and SGA neonates are prone to hypoglycemia due to their limited glucose storage, insufficient adaptive changes, and underdeveloped metabolic pathways.[6]

A previous study reported lower cord blood glucose levels in Caesarean-delivered newborns.[7] However, we found no significant difference in Caesarean-delivered vs. vaginally-delivered newborns for the two groups. This finding might have been due to the limited sample size of our study and the different method of measuring the blood glucose level as we took the samples from peripheral blood specimen.

Of hypoglycemic infants, 48.8% had birth asphyxia. The risk of developing hypoglycemia increased three-fold compared to vigorous-born infants. A previous reported an incidence of 26.86% hypoglycemia among infants born with birth asphyxia.[8] During asphyxia, an increased anaerobic glycolysis rate along with glycogenolysis predisposes neonates to hypoglycemia.[9]

The main limitation of this study was its susceptibility to information bias, as data collected reflected what was recorded in the medical charts. Many factors may affect the prevalence of neonatal hypoglycemia in different medical centers. Thus, performing a hospital-based study in this regard is important to establish comprehensive neonatal care guidelines to reduce neonatal mortality and morbidity caused by hypoglycemia among newborns at risk.

In conclusion, prematurity and low birth weight are significant risk factors associated with neonatal hypoglycemia. Routine screening and monitoring of blood glucose is recommended for preterm newborns and infants with low birth weight.

Conflict of Interest

None declared.

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Original Article

Clinicopathologic and molecular profiles of Duchenne and Becker muscular dystrophy

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Abstract

Background Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are allelic X-linked recessive diseases caused by mutations in the dystrophin (DMD) gene. To our knowledge, molecular analysis to differentiate between DMD and BMD has never been performed in Indonesia.

Objective To elaborate the clinicopathologic and molecular profiles of DMD/BMD patients in Yogyakarta, Indonesia.

Methods Eighteen muscle biopsy specimens of patients clinically suspected to have DMD/BMD were collected. Possible associations of clinical manifestations, histopathological grading, and immunohistochemistry (IHC) results were analyzed. Polymerase chain reaction (PCR) was performed to identify mutations in exon 52.

Results Positive Gower's sign and high serum creatine kinase (CK) were observed in most patients. The IHC of dystrophin in two female patients suggested that they were manifesting carriers. Of the 16 male patients, 12 showed negative IHC staining, indicating DMD, while 4 patients demonstrated weak expression of dystrophin, indicating BMD. There was a significant association between high CK level and IHC results (P=0.005), indicating higher CK level in DMD patients. Histopathological grading of muscle biopsy was significantly associated with diagnosis of DMD/BMD using IHC (P=0.01), showing more severe tissue damage in DMD patients. None of the subjects had the single exon 52 deletion.

Conclusion This is the first report of a clinicopathologic and molecular profile of DMD/BMD in an Indonesian population. Serum CK level and histopathological grading of muscle biopsy are useful in distinguishing DMD from BMD in settings where an IHC assay is not available. **[Paediatr Indones. 2019;59:257-64; doi: http://dx.doi.org/10.14238/pi59.5.2019. 257-64**].

Keywords: *dystrophin gene; DMD; BMD; CK; immunohistochemistry*

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are recessive, X-linked, hereditary diseases due to mutations in the dystrophin (DMD) gene. Duchenne muscular dystrophy is the most common type of muscular dystrophy, affecting 1/3,500 male births.[1] This disease is characterized by progressive muscle weakness from childhood and loss of ambulation prior to the age of 12 years. Patients generally die due to respiratory or cardiac failure before their third decade of life. BMD has similar clinical features to DMD, however, it has slower disease progression. Patients may able to walk until 16 years of age. BMD patients also have better quality of life and longer life expectancy compared to DMD patients.[1] Early detection of DMD and BMD is possible, even before muscle weakness or clinical manifestations appear, as they are marked by increased serum CK level from the early years of life.[2]

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The dystrophin gene is one of the largest in the human genome, with a size of more than 3 Mb on the X chromosome and 79 exons. Dystrophin codes for a 14kb mRNA which is translated into the dystrophin protein. Dystrophin and other glycoproteins in the cell membrane form a dystrophin-glycoprotein complex (DGC), which has the function of stabilizing muscle fiber membranes. In DMD, dystrophin is completely absent, resulting in progressive muscle weakness which eventualy leads to premature death of the patient. In BMD patients, a partially functional protein is still produced, so the clinical manifestations are less severe compared to those of DMD patients. BMD patients have a higher life expectancy.[3-6] The DMD/BMD screening can be performed by blood serum CK measurements. Gower's sign and waddling gait in males with positive family history could prompt physicians to perform further examinations to diagnose or rule out DMD/BMD.[7]

The DMD/BMD clinical progress can be predicted by the disruption pattern caused by mutation(s) in the dystrophin gene. Mutations may alter the mRNA reading frame to in-frame (partially functional protein), or out-frame (absolutely absent protein).[8] One of the most promising strategies to correct the absence of dystrophin production in DMD is exon-skipping therapy, which involves changing an out-frame mutation to an in-frame one using antisense oligonucleotides or small molecules, so that the patient would have a less severe phenotype, like that of BMD.[9,10]

Immunohistochemical staining of muscle biopsy specimens and genetic analysis to detect mutations in the dystrophin gene are the gold standard tests to diagnose DMD/BMD. However, both of these examinations have not been performed regularly in Indonesia. Thus, robust data regarding clinical characteristics, epidemiology, and molecular profiles of DMD/BMD patients in Indonesia are currently unavailable. Future therapy using 'exon skipping' or 'stop codon read through' strategies will require detailed patient information on molecular and mutational status. Hence, we aimed to determine the clinical, histopathological, and molecular characteristics of DMD/BMD patients in Indonesia by conducting various tests on their muscle biopsy specimens in Sardjito General Hospital, Yogyakarta.

Methods

This observational analytical study had a cross-sectional design. There was no follow-up or intervention for the study subjects. Subjects were patients with clinically-suspected DMD/BMD in Sardjito General Hospital from January 2010 to December 2015, who had undergone muscle biopsy. Muscle biopsy specimens were in formalin-fixed, paraffin-embedded (FFPE) form. To determine the histopathologic grade of dystrophin in the muscle, the FFPE muscle biopsy was sliced and stained with hematoxylin-eosin (HE) for histopathological assay. The samples were also immunostained with dys-2 antibody (*Leica Biosystem*, Newcastle, USA) to detect dystrophin expression, determining whether patient was DMD, BMD or none of them. The pathologist, an expert in muscle histopathology and dystrophin immunostaining, performed the histopathological analysis. Based on histologic appearance, muscle biopsy is categorized into 4 grade. Grade 1 is characterized by retention of fascicular pattern of muscle fibers with no obvious fibrosis and fat infiltration, grade 2 is demonstrated by retention fascicular pattern of muscle fibers with minimal fibrosis and/or fat infiltration, grade 3 is shown by disrupted muscle fibers with marked fibrosis and/or fat infiltration, meanwhile grade 4 is indicated by severe change of muscle fibers with more than 50% fat and fibrosis replacement.[11] Dystrophin immunostaining is interpreted by strong membrane expression of dystrophin in normal control, completely no expression in DMD patients and focally expressed in BMD patients.[12]

The DNA extraction from all FFPE specimens was performed on those showing negative or partially positive immunohistochemical (IHC) results. The DNA extraction was conducted using a GeneJET FFPEDNA extraction kit (*Thermoscientific Carlsbad*, CA, United States). Polymerase chain reaction (PCR) was performed using PCR KAPA Taq ReadyMix (*Kapa Biosystems*, Boston, Massachusetts, United States). The PCR mixtures consisted of KAPA Taq master mix, specific primers of exon 52, and a DNA template in a total volume of 20 ul after addition of H_2O. Exon 52 was amplified using the following specific primer sequences: DMD_52F GTG TTT TGG CTG GTC TCA CA and DMD_52R ATG GAC TGA AAA TCT CAG CAC AAT (expected amplicon size

366bp). DNA was denaturated at 95°C for 3 minutes. Thirty-five PCR cycles were performed as follows: 30 seconds at 93°C (denaturation), 30 seconds at 61.2°C (annealing), and 1 minute at 72°C (elongation). The PCR products were subjected to electrophoresis on a 2% agarose gel, visualized by ethidium bromide fluorescence, and photographed. All specimens were assayed with positive and negative controls.

Research data were analyzed using suitable statistical analyses with *SPSS 23.00* software. Mann-Whitney test was used to analyze for an association between serum CK increase and IHC results. Fisher's exact test was used to analyze for an association between histopathological degree in DMD and BMD patients. This research was approved by the Research Ethics Committee of the Faculty of Medicine, Gadjah Mada University.

Results

From January 2010 to December 2015, 115 patients were clinically diagnosed with DMD/BMD in Sardjito General Hospital. However, only 18 patients had undergone gastrocnemius muscle biopsy to establish the diagnosis histopathologically. The age range of those 18 patients was 3 to 22 years, with mean age of 9.6 years. Among the 18 patients, 2 were female. Demographic and clinical characteristics of subjects are summarized in **Table 1**. All patients in this study had positive Gower's sign during neurological examination. Increased serum CK level was found in 16/18 patients, while 14/18 showed abnormal gait such as waddling or walking on tiptoe. Gastrocnemius muscle pseudohypertrophy was observed in 11/18 patients. Skeletal abnormalities (such as scoliosis, lordosis) and drop foot were seen in 3/18 patients. Cardiomyopathy occurred in 2/18 patients, while mental retardation was obeserved in 1/18 patient. Three patients had positive familial history of muscle weakness. Increased serum CK level was detected in 16/18 patients (CK data for one patient was not available). Subjects' mean CK level was 10,601 U/L, ranging from 190 U/L to 91,746 U/L. Electroneuromyography (ENMG) examination was performed in 11/18 patients, 8/18 of whom were diagnosed with myopathy, and 3/18 of whom were diagnosed with neuropathy.

Table 1. Clinical characteristics of DMD/BMD patients

Characteristics	N	Mean (SD)
Age, years		9.61 (4.15)
0-5	2	
6-10	9	
11-15	6	
16-20	0	
>20	1	
Sex		
Male	16	
Female	2	
Clinical features		
Gower's sign	18	
CK ↑	16	
Abnormal gait	14	
Gastrocnemius pseudohypertrophy	11	
Family history	3	
Bone abnormality	3	
Cardiomyopathy	2	
Mental retardation	1	
CK level, U/L		10,600.5
Myopathy	8	(21,461.6)
Neuropathy	3	
ENMG		
Myopathy & neuropathy	11	
N/A	7	

Muscle biopsy samples were histopathologically assessed using standard HE staining by a trained pathologist (**Figure 1**). The specimens showed variations in muscle fiber diameter size, increased centrally-located nuclei, necrosis, lymphocyte infiltration, fat replacement, and fibrosis in 18/18, 14/18, 13/18, 17/18, 18, and 15/18 patients, respectively (**Table 2**). Grading for muscle damage was performed based on fibrosis and fat infiltration in muscle fibers.11 Four of 18 patients showed a lower degree (grade 2), while 14/18 patients showed higher degrees of grades 3 (8/18 subjects) and 4 (6/18 subjects) (**Table 3**).

Immunohistochemistry (IHC) test results of muscle biopsy specimens are shown in **Figure 2**. Six

Table 2. Histopathological profiles - HE staining (N=18)

Histopathological feature	n
Various diameters of muscle fibers	18
Fibrosis	15
Fat infiltration	18
Increased intralocated nuclei	14
Necrosis	13
Lymphocyte infiltration	17

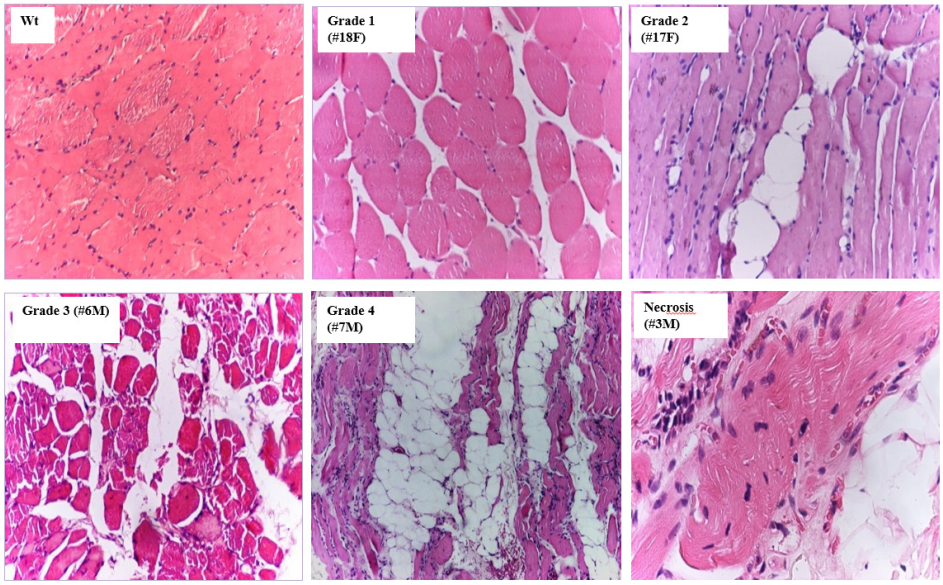

Figure 1. Muscle biopsy grading. Gastrocnemius biopsy with HE staining in wild type (Wt), BMD (#18F, #17F), and DMD (#6M, #7M, #3M) patients. Wt=muscle fibers in fascicular arrangement, uniform muscle fiber diameters, peripherally-located nuclei, and without lymphocyte infiltration, fat infiltration, fibrosis, or necrosis. DMD/BMD=variable muscle fiber diameters, increased centrally-located nuclei, lymphocyte infiltration, fibrosis, and fat infiltration, categorized as grades 1, 2, 3, and 4.

patients expressed the dystrophin protein focally in some muscle fibers, 4 of whom were male, confirming a BMD diagnosis; 2 patients were female, indicating carrier status. The muscle biopsy specimens from the remaining 12 patients showed no dystrophin expression, confirming a DMD diagnosis (**Table 4**). Correlation between histopathological degree and dystrophin IHC. **Table 5** shows that 6/18 patients with grade 4 had DMD and 2/18 had BMD. Among the 8 patients with grade 3, 7/8 had DMD and 1/8 had BMD. Two patients with grade 2 were female, with focal expression of dystrophin in some muscle fibers, suggesting manifestation of carrier status. The

Table 4. Dystrophin immunohistochemistry based on sex (N=18)

Dystrophin IHC	Sex	n	Diagnosis
Negative	Male	12	DMD
	Female	0	(-)
Focal positive	Male	4	BMD
	Female	2	Carrier

Table 3. Histopathological grading (N=18)

	Grading	n
Grade 1	Retention fascicular pattern of muscle fibers with no obvious fibrosis and fat infiltration	0
Grade 2	Retention fascicular pattern of muscle fibers with fibrosis and/or fat infiltration	4
Grade 3	Disrupted muscle fibers with marked fibrosis and/or fat infiltration	8
Grade 4	Severe change of muscle fibers with more than 50% fat and fibrosis replacement	6

Table 5. Histopathological grading and dystrophin IHC result (n=18)

Histopathological grading	BMD	DMD	Carrier
Grade 1			
Grade 2	2		2
Grade 3	1	7	
Grade 4	1	5	

Table 6. Association between histopathological degree and diagnosis of DMD/BMD (N=16)

Histopathological grading	BMD	DMD	P value
Grade 1 and 2	2	0	0.01
Grade 3 and 4	2	12	

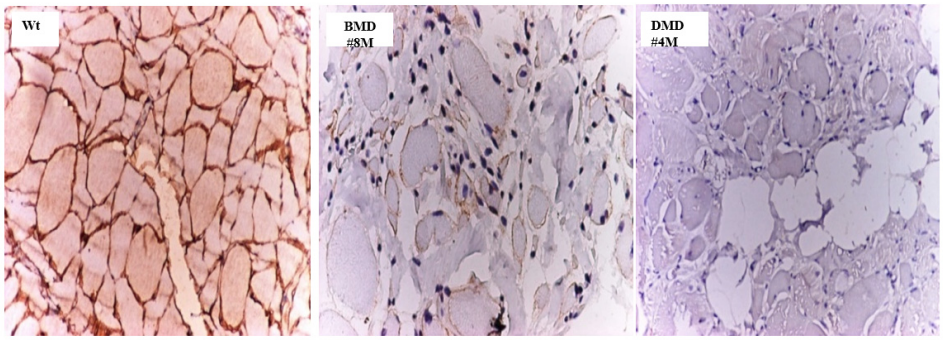

Figure 2. Dystrophin immunostaining (IHC). Dys-2 immunostaining was performed in wild type (Wt), BMD (#8M), and DMD (#4M) patients. Wt=dystrophin was strongly expressed in muscle fiber membranes, giving a spider web appearance. BMD=dystrophin was focally expressed in some muscle fibers due to inframe mutation in the dystrophin gene. DMD=dystrophin expression was completely absent due to outframe mutation in the dystrophin gene.[12]

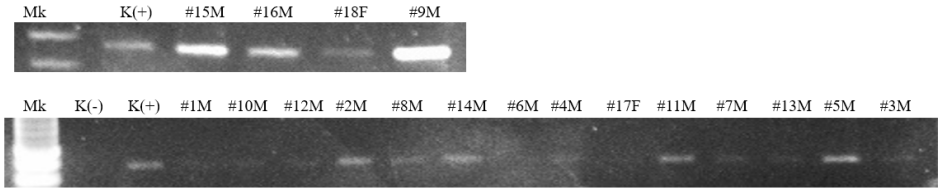

Figure 3. Gel electrophoresis of the PCR analysis. Exon 52 deletions were not observed in all DMD/BMD patients, since PCR amplification of exon 52 was detected in all specimens. (Mk= marker, K= control).

mean age of DMD patients based on dystrophin IHC results was 8.5 years, while the mean age of BMD patients was 11.4 years. However, this age difference was not statistically significant (P=0.18). Mann-Whitney test revealed that serum CK level was significantly higher in DMD than in BMD patients, based on IHC results (P=0.005). Fisher's exact test showed that histopathological degree was significantly associated with a diagnosis of DMD/BMD using IHC (P=0.01, **Table 6**), as patients with grades 3 and 4 were more likely to be diagnosed with DMD in IHC test, while grades 1 and 2 were more likely to be diagnosed with BMD. Mutation analysis (**Figure 3**) showed that the exon 52 deletion was not found in any of our patients. This analysis was confirmed by comparing all the samples to wild type (positive control) amplification.

Discussion

A DMD/BMD diagnosis is established based on genetic analysis or dystrophin staining of muscle biopsy specimens.[7,13] There were 115 patients clinically diagnosed with DMD/ BMD in Sardjito General Hospital, Yogyakarta in year 2010-2015. However, only 18 patients had undergone gastrocnemius muscle biopsy to establish the diagnosis histopathologically. Genetic analysis was not performed. In Indonesia, diagnosis of the disease is still based on clinical manifestations, which cannot be used to differentiate DMD from BMD or other muscular dystrophies.

There were 2 female patients with muscle weakness among our 18 subjects. Since females have two X chromosomes, theoretically they are carriers of the dystrophin gene mutation, and generally have no clinical manifestations. However, some manifesting carriers have been reported previously, with mild to severe clinical features.[14-20] Clinically, these two female subjects showed muscle weakness with positive

Gower's sign, but CK level was slightly elevated. One girl had a family history of muscle weakness. Both subjects had histopathological grade 2 muscle damage and IHC-demonstrated focal dystrophin expression in some muscle fibers. Explanations on how carriers may show clinical symptoms include (1) mutations in both alleles of the Xp21-chromosome,16 (2) loss of one X chromosome, e.g., in Turner syndrome,21 (3) abnormal X chromosome, e.g., deletion, duplication, or translocation,[21] and (4) extremely skewed X chromosome inactivation.[22]

Subjects' mean age was 9.6 years; the oldest patient (22 years) was diagnosed with BMD based on IHC results. In agreement with previous studies, DMD generally has an early onset and severe phenotype, while BMD has a late onset with milder phenotype and longer life expectancy.[1,23] All subjects had positive Gower's sign, and most had high serum CK level, both of which are valuable clinical markers in suspecting DMD/BMD.[7,13] Other symptoms such as abnormal gait, muscle weakness, pseudohypertrophy, skeletal abnormality, cardiomyopathy or mental retardation should also be carefully observed to support the diagnosis of DMD/ BMD.[8, 24-26]

The supporting ENMG examination revealed that 8/18 patients had muscle abnormality or myopathy. Electrodiagnostic studies have an important role in evaluating patients suspected of having myopathy. This examination can be used as an alternative diagnostic tool in confirming muscle abnormality, narrowing the differential diagnosis and identifying the best muscle biopsy location. Needle electromyography is the most informative tool to assess spontaneous muscle activity and motor unit action.[27]

In DMD/BMD patients, dystrophin gene mutations cause dystrophin loss, affecting DGC complex function in stabilizing cell membrane, which makes cells susceptibile to injury. Inflammatory mediators are attracted to injury sites, causing continuous chronic inflammation, necrosis, and replacement of muscle fibers with fat tissue and fibrosis.[23] Muscle abnormalities appear as varied sizes of muscle fibers, increased centrally-located nuclei, necrosis, lymphocyte infiltration, fibrosis, and fat replacement (pseudohypertrophy).[11,28,29]

In our study, patients diagnosed with DMD by IHC showed higher serum CK levels compared to BMD patients. This finding was in agreement with previous studies showing that more severe muscle damage occurred in DMD patients compared to BMD patients. The DMD patients also had higher CK levels with early onset of age 1-6 years (peak of 3-5 years), with a 0.18 U/L/year rate of decline. The BMD patients had later onset compared to DMD patients, generally at 10-15 years of age, with a slower rate of decline of 0.06 U/L/year. These reports indicated that the loss of muscle mass was more progressive in DMD patients.[2,30]

In our study, histopathological grading was significantly correlated with diagnosis of DMD/BMD using IHC. Patients with grades 3 and 4 were more likely to be diagnosed with DMD by IHC test, while patients with grades 1 and 2 were more likely to be diagnosed with BMD. To our knowledge, studies of this type have not been performed in Indonesia. Histopathological grading of muscle damage was introduced by Kinali *et al.*[11] to compare severity of muscle damage in DMD patients with magnetic resonance imaging (MRI) results. Our study shows that histopathological grading using the method introduced by Kinali *et al*. can be used to distingush DMD from BMD. This result may help many hospitals without IHC-staining facilities to diagnose DMD/ BMD.

Genetic analysis of DMD gene exon 52 showed no exon deletion in any of the 18 subjects. Takeshima *et al*.[1] reported that deletion of exon 52 is the most common single exon deletion. The small sample size of our study may be the reason for this discrepancy. Analysis on other exons should be performed to identify possible mutations in other regions of the dystrophin gene. Further study using a larger sample size is also needed to understand the characteristics of this disease in Indonesian populations.

In conclusion, this is the first report of clinicopathological and molecular profiles of DMD/ BMD in Indonesian population. The IHC and genetic analysis are standard tools to diagnose DMD and BMD. Serum CK level and histopathological grading of muscle biopsy are useful in distinguising DMD from BMD in setting where IHC analysis is not available. In this study, single deletion of exon 52 was not found in small size of Indonesian population.

Conflict of Interest

None declared.

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Original Article

Waist circumference and waist-hip ratio as screening tools for hypertension in children aged 6-11 years

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Abstract

Background Hypertension in children is associated with obesity. The renin-angiotensin-aldosterone system has been associated with intra-abdominal fat tissue. Anthropometric parameters for determining nutritional status include waist circumference and waist-hip ratio. Many studies have shown that waist circumference and waist-hip ratio are more precise to determine overweight or obese.

Objective To determine the usefulness of waist circumference and waist-hip ratio as hypertensive screening tools for children aged 6-11 years.

Methods This analytical study with cross-sectional design and multistage cluster sampling method was conducted in August-September 2017 at a primary school in Bandung, West Java, Indonesia. Subjects underwent height, weight, waist circumference, hip circumference, and blood pressure measurements. Receiver operating characteristic (ROC) curve analysis was done to obtain the area under curve (AUC), cut-off point, sensitivity, specificity, and prevalence ratio.

Results Subjects were 325 children consisting of 187 males and 138 females. Hypertension was diagnosed in 47 children (37 males and 10 females). Mean waist circumference and waist-hip circumference ratio were significantly higher in the hypertensive group than in the normotensive group. The hypertensive group had a mean waist circumference of 72.6 (SD 12.8) cm, AUC 0.779 (95%CI 0.730 to 0.823; P<0.001), cut-off point >65 cm, sensitivity 66.0%, specificity 76.3%, and prevalence ratio 4.55. This group had mean waist-hip ratio of 0.94 (SD 0.10), AUC 0.724 (95%CI 0.672 to 0.772; P<0.001), waist-hip ratio cut-off >0.91, sensitivity 59.6%, specificity 77.0%, and prevalence ratio 3.73.

Conclusion Waist circumference >65 cm or waist-hip ratio >0.91 can be used to screen for hypertension in children aged 6-11 years with negative predictive values of 92.0% and 91.8%, respectively. [Paediatr Indones. 2019;59:265-70; doi: http://dx.doi.org/10.14238/pi59.5.2019.265-70].

Keywords: *hypertension; waist circumference; waist-hip ratio*

The prevalence of hypertension in children and adolescents has increased in recent years, possibly related to the increased prevalence of obesity in children and adolescents.[1,2] Hypertension in children frequently has no symptoms so it often goes undiagnosed. Hypertension in adults begins in childhood.[1] Childhood hypertension increases the risk of cardiovascular disease, stroke, and death from cardiovascular disease during adulthood.[3,4] Likewise, childhood obesity increases the risk of cardiovascular disease in adulthood.[5]

Studies have shown that overweight or obese children are at risk of hypertension.[6,7] The renin-angiotensin-aldosterone system has been associated with intra-abdominal fat tissue compared to other sites, and may ultimately have an effect on blood pressure elevation.[8-11] This association between intra-abdominal fat tissue and the renin-angiotensin system has led some researchers to conclude that the anthropometric parameters of waist circumference and waist-hip ratio more precise determining overweight or obese nutritional status than other measurements.[5,7,12]

Various studies have been conducted to determine the relationship between body mass index and the

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incidence of hypertension in children aged 12 years or more.[13] Blood pressure in children aged 6-7 years was considered not to change more until adulthood,13,14 if hypertension occurs, it will known immediately, so our study was done in children aged 6-11 years. The purpose of this study was to evaluate waist circumference and waist-hip ratio as hypertensive screening tools for children aged 6-11 years.

Methods

The inclusion criteria were children aged 6 years 0 month to 11 years 1 month, and written informed consent from parents/guardians. Exclusion criteria were children with known kidney disease, hypertension, congenital heart disease, or hormonal abnormalities, as well as those who received corticosteroid medications, had a family history of hypertension, or low birth weight. The selection of study subjects was done gradually with a multi-stage cluster sampling method based on the population of a primary school in Bandung, West Java.

Subjects underwent body height, body weight, waist circumference, hip circumference, and blood pressure measurements which were performed by physicians at school in the morning hours (8.00 am to 11.00 am). Blood pressure was measured three times with a 5 minute interval between each measurements. Before the blood pressure measurement, subjects were asked to sit still for 5 minutes. Body height were measured using a portable stadiometer to the nearest 0,1 cm and weight were measured with a balance beam scale (SECA measuring equipment) to the nearest 0,1 kg. To determine nutritional status, we used the *2000 Center for Disease Control and Prevention* (CDC) chart,[15] while blood pressure interpretation was based on *The National High Blood Pressure in Children and Adolescents* tables.[4,16]

The unequal numerical difference test was used to analyze waist circumference and waist-hip ratio usefulness as screening tools for hypertension in children. Data normality was analyzed using Kolmogorov-Smirnov test. Unpaired t-test was used for normally-distributed data and Mann-Whitney test was used for non-normally distributed data. Receiver operating characteristic (ROC) curve analysis was used to obtain area under the curve (AUC), as well as determine cut-off points, sensitivity, specificity, and prevalence ratio for waist circumference and waist-hip ratio. All analyses were processed with *SPSS for Windows version 21.0* software. Results with P values ≤0.05 were considered to be statistically significant

This study was approved by the Health Research Ethics Commission of the Ministry of Research, Technology, and Higher Education Universitas Padjadjaran Medical School, Bandung.

Results

During the August-September 2017 study period, there were 329 children aged 6-11 years at the primary school. One child was excluded because of previously diagnosed hypertension and taking routine medication, 2 children because of premature birth, and 1 child because of nephrotic syndrome and taking routine prednisone, so the total number of subjects was 325. The characteristics of the study subjects are shown in **Table 1**. Of the 325 subjects, 47 (14.5%) had hypertension and 278 had normal blood pressure. Children with obese nutritional status had a higher percentage of hypertension compared to those with overweight, normal, and underweight nutritional status.

The analysis of relationships between hypertension and waist circumference as well as waist-hip ratio are shown in **Table 2**. Higher waist circumference and waist-hip ratio had significant associations with hypertension.

To determine the use of waist circumference and waist-hip ratio as screening tools for hypertension in children, ROC analysis was performed to assess the AUC as shown in **Table 3**. A waist circumference cut-off point of >65 cm was most valid with NPV of 92%. A waist-hip ratio cut-off point of >0.91 was most valid, with NPV of 91.8%.

The hypertension prevalence ratios based on waist circumference and waist-hip ratio is shown in Table 4. Children with a waist circumference >65 cm potentially have a 4.55 times higher risk of hypertension compared to those with waist circumference <65 cm. In addition, children with waist-hip ratio >0.91 have a 3.73 times higher risk of hypertension compared to those with waist-hip ratio <0.91.

Table 1. Characteristics of study subjects

Characteristics	Total (N=325)	Hypertensive (n=47)	Normal (n=278)
Sex, n (%)			
Male	187 (57.5)	37 (19.8)	150 (80.2)
Female	138 (42.5)	10 (7.2)	128 (92.8)
Mean age (SD), years	9.3 (1.8)	10.0 (1.1)	9.2 (1.9)
Age range, years	6-11	7- 11	6-11
Age by year, n (%)			
6	35 (10.8)	0 (0.0)	35 (100.0)
7	34 (10.5)	1 (2.9)	33 (97.1)
8	50 (15.4)	4 (8.0)	46 (92.0)
9	32 (9.8)	12 (37.5)	20 (62.5)
10	28 (8.6)	9 (32.1)	19 (67.9)
11	146 (44.9)	21 (14.4)	125 (85.6)
Mean height (SD), cm	134.5 (12.5)	139.9 (10.3)	133.6 (12.6)
Height range, cm	103-164	117-162	103-164
Nutritional status, n (%)			
Underweight	57 (17.5)	6 (10.5)	51 (89.5)
Normal	193 (59.4)	15 (7.8)	178 (92.2)
Overweight	44 (13.5)	12 (27.3)	32 (72.7)
Obese	31 (9.5)	14 (45.2)	17 (54.8)

Table 2. Analysis of waist circumference and waist-hip ratio to hypertension

Variables	Total (N=325)	Hypertensive (n=47)	Normal (n=278)	P value
Waist circumference, cm				
Mean (SD)	62.1 (10.4)	72.6 (12.8)	60.3 (8.8)	<0.001*
Range	45.0-100.0	53.0-100.0	45.0-87.0	
Waist-hip circumference ratio				
Mean (SD)	0.88 (0.08)	0.94 (0.10)	0.87 (0.07)	<0.001*
Range	0.70-1.30	0.78-1.30	0.70-1.20	

*T-test

Table 3. ROC analyses of waist circumference and waist-hip ratio against hypertension

Variables	AUC (95% CI)	Cut-off point	P value	
Waist circumference, cm	0.779 (0.730 to 0.823)	>65	<0.001	Sensitivity= 66.0% Specificity = 76.3% PPV = 32.0% NPV = 92.0%
Waist-hip circumference ratio	0.724 (0.672 to 0.772)	>0.91	<0.001	Sensitivity = 59.6% Specificity = 77.0% PPV = 30.4% NPV = 91.8%

PPV= positive predictive value, NPV=negative predictive value

Table 4. Prevalence of waist circumference and waist hip ratio to hypertension

Variables	n	Hypertension (n=47)	Normal (n=278)	Prevalence ratio (95% CI)	P value
Waist circumference					
≥65 cm	97	31 (32.0)	66 (68.0)	4.55 (2.63 to 7.88)	<0.001
<65 cm	228	16 (7.0)	212 (93.0)		
Waist-hip ratio					
≥0.91	92	28 (30.4)	64 (69.6)	3.73 (2.20 to 6.30)	<0.001
<0.91	233	19 (8.2)	214 (91.9)		

Discussion

In children aged 6-11 years, waist circumference and waist-hip ratio was significantly higher in the hypertensive group than the normotensive group (P<0.001). Previous studies have also shown a correlation between waist circumference and hypertension.[8,17-20] Waist circumference is a reflection of morbidity in obesity and closely related to levels of intra-abdominal fat.[3] Waist circumference and waist-hip ratio represent the distribution of body fat, both intracutaneously and intra-abdominally.[5,14,17,18,22] Intra-abdominal fat is thought to produce mineralocorticoid-releasing factor that induces aldosterone synthesis. Aldosterone increases blood pressure through mineralocorticoid receptors located in various tissues such as the kidneys and vasculature,[5,10,11] so measurement of waist circumference and waist-hip ratio should be considered as screening tools to predict hypertension in children.

Using ROC curve analysis, the waist circumference AUC was 77.9% (95%CI 0.730 to 0.823; P<0.001) indicating a moderate level of accuracy. The AUC value of 77.9% means that if waist circumference is used to predict hypertension in children there are 77/100 children who have hypertension. The waist circumference cut-off point was >65 cm, with PPV 32.0% and NPV 92.0%. The waist circumference can be used as a tool to predict probability of a healthy child/normal blood pressure. The waist-hip ratio cut-off point of >0.91 had a PPV of 30.4% and NPV of 91.8%. This finding suggests that waist-hip ratio can be used as a tool to predict the true likelihood of a healthy child/normal blood pressure (among all children who show normal blood pressure). With the negative predictive value, waist circumference and waist-hip ratio can be used as a screening tool for the possibility of hypertension.

The results of this predictive value validity study were similar to those of another study with increased waist circumference correlating to the incidence of hypertension and metabolic disorders such as hyperlipidemia, ,in which waist circumference cut-off point of 59 cm for boys (PPV 34.5% and NPV 86.9%) and 57 cm for girls (PPV 22.6% and NPV 90.9%).18 We were unable to determine the cut-off points on the basis of sex, as the number of subjects was not as large and we had unequal numbers of males and females.

A previous study reported that waist circumference with percentile > 90 correlated to the occurrence of hypertension.[22] However, another study differed slightly, concluding that increased waist circumference correlated significantly with an increase in BMI and increased systolic or diastolic blood pressure. But increased waist circumference showed a weak correlation to elevated triglycerides or total cholesterol. In addition, the waist-hip ratio did not correlate with an increase in BMI, and only slightly correlated to the incidence of hypertension as well as increased triglycerides or total cholesterol.[17]

The prevalence of hypertension in our study was high at 14.5%, which differed from the 2-5% prevalence of hypertension in children estimated by the *World Health Organizatio*n (WHO).[1,18] Studies that calculated weight and height reported hypertension prevalence of 4.5%.[14,19]

In our study, the prevalence ratio for waist circumference >65 cm was 4.55, indicating that children with waist circumference >65 cm had 4.55 times risk of hypertension compared to those with waist circumference <65 cm. We also noted a prevalence ratio of 3.73 for waist-hip ratio >0.91, indicating that children with a waist-hip ratio >0.91 had 3.73 times the risk of hypertension compared to those with waist-hip ratio <0.91. As such, parents of

children who have waist circumference >65 cm or waist-hip ratio >0.91 should be aware of the possibility of hypertension in future life. Various efforts should be made to prevent the occurrence of hypertension in children, such as a good diet, weight reduction, and regular exercise.

Waist circumference and waist-hip ratio in our study showed the highest validity of the NPV, whereas sensitivity, specificity, and PPV were not as high. To further assess the validity of the latter parameters, further study is needed with a larger sample size. Studies should be done from different regional and ethnic areas so that these parameters can be thoroughly explored.

In conclusion, waist circumference cut-off point >65 cm and waist-hip ratio cut-off >0.91 in children aged 6-11 years can be used as hypertensive screening tools with negative predictive values of 92.0% and 91.8%, respectively.

Conflict of Interest

None declared.

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Original Article

Increased lipoxin B4 levels in children with atopic dermatitis

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Abstract

Background Atopic dermatitis (AD) is the most common chronic inflammatory skin disease in the pediatric population. The pathophysiology of AD is complex and not clearly understood. The role of lipoxin B4 (LXB4), an anti-inflammatory mediator, has not been sufficiently investigated in children with AD to our knowledge.

Objective To compare the levels of serum LXB4 between children with AD and healthy controls.

Methods Three groups of children were enrolled in this study: a SPT-Pos group (skin prick test positive 21 subjects with AD), a SPT-Neg group (skin prick test negative 22 subjects with AD), and a control group (23 healthy subjects). Subjects' serum LXB4 levels of were measured with an ELISA technique. Also, eosinophil counts and total immunoglobulin E (IgE) levels were compared among all groups.

Results We observed significantly higher LXB4 levels in AD patients than in controls. Also, LXB4 levels were significantly higher in the SPT-Pos group than in the SPT-Neg group and control group. However, no significant difference was observed between the SPT-Neg and control groups.

Conclusion The LXB4 may have an anti-inflammatory mediator role in the pathogenesis of AD in children. The LXB4-associated pathways may be considered in the development of novel therapeutic approaches for the treatment of patients with AD. [Paediatr Indones. 2019;59:271-5; doi: http://dx.doi.org/10.14238/pi59.5.2019.271-5].

Keywords: *lipoxin B4; atopic dermatitis; LXB4; skin prick test*

Atopic dermatitis is one of the most common chronic inflammatory skin diseases affecting 15-20% of children worldwide.[1] The pathogenesis of AD is not clearly understood. However, complex interactions between environmental exposure and genetic factors are important in the pathogenesis of AD.[2] The role of eosinophils in the pathogenesis of AD also has not been clearly understood. It seems possible that eosinophils contribute to host defense against invading microbes through the defective skin barrier by generating eosinophil extracellular DNA traps which regulate immune responses.

Approximately 80 % of AD patients have increased total and specific IgE to allergens, especially food allergens. Atopic dermatitis is the first manifestation of allergic march preceding respiratory allergic diseases, such as asthma and allergic rhinitis in most children.[3] Recently, a study that focused on

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resolution of inflammatory processes gave rise to the identification of anti-inflammatory mediators such as lipoxins (LXs, i.e., LXA4 and LXB4).[4] The LXs are produced by membrane phospholipids during cell–cell interactions via 15-lipoxygenase and 5-lipoxygenase enzymes. As an anti-inflammatory mediator, LXs have been associated with some chronic inflammatory diseases, such as rheumatoid arthritis, sarcoidosis, and asthma.[5] In allergic diseases, the presence of LXs have been demonstrated in the upper and lower respiratory tracts in nasal lavage fluid and in bronchoalveolar lavage fluid, respectively.[6,7] In an animal study, LXB4 significantly decreased airway inflammation, nasal mucosal leukocytes as well as decreased IgE-mediated mast cell and eosinophil degranulation. Also, LXB4 decreased eotaxin-dependent eosinophil chemotaxis and expression of type 2 cytokine receptors which have some different roles in the pathogenesis of AD.4 A role for LXB4 has not been investigated in patients with AD, to our knowledge.

The aim of this study was to investigate serum LXB4 levels among SPT-Pos, SPT-Neg, and healthy controls.

Methods

This prospective study was performed at the Maternity and Children's Hospital, Batman, Turkey. Atopic dermatitis was diagnosed based on standard Hanifin and Rajika criteria's in this study.[8] A total of 66 children aged 3 months - 3 years were enrolled in the study. Forty-three patients with AD were divided into either the SPT-Pos (21 subjects) or the SPT-Neg group (22 subjects). The control group comprised 23 healthy children. Children with infectious diseases such as upper or lower airway infections within the four weeks prior to the study were excluded. At presentation, children underwent skin prick testing (SPT) on the upper back. The SPT kit (*Allergopharma*, Reinbek, Germany) tested 10 antigens including two aero-allergens (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cow's milk, egg white, egg yolk, soy, nut, peanut, wheat, and tuna), with positive and negative controls, histamine and serum physiologic, respectively. Reactions were considered to be positive if an induration >3 mm was observed, compared to the negative control. Total IgE levels were measured with a nephelometric method. Eosinophil counts were determined from Coulter counter leukocyte measurements. Serum LXB4 levels were measured using an LXB4 ELISA kit (*E2264Ge, EIAab Company*, United Kingdom), according to the manufacturer's instructions

Data were analyzed using *SPSS version 22* software (*IBM*, USA). Shapiro-Wilk test was carried out to determine the normality of data distribution and revealed abnormal data distributions for LXB4 levels, total IgE, and eosinophil counts ($P<0.05$). Hence, median values and minimum-maximum ranges were determined, and all groups were compared by Kruskal-Wallis test. We also used a post-hoc Bonferroni modified Mann-Whitney U test for binary comparison. Correlation analysis was performed with Spearman's correlation test. Results with P values <0.05 were considered to be statistically significant.

This study was approved by the ethic comity of Batman District State Hospital in Batman, Turkey. Written informed consent was acquired from the parents of all participating children

Results

Patients' characteristics and median total IgE, eosinophil counts, and LXB4 values are shown in **Table 1**. Of 43 patients with AD, 21 in the SPT-Pos group had positive SPT, median age 8 months (range 3-24 months), with 9 males and 12 females. Of the SPT-Pos patients, 10 had egg white allergy, 9 had cow's milk allergy, and 2 had multiple food allergy (one had cow's milk and egg white; one had egg white and wheat). The remaining 22 with AD in the SPT-Neg group had negative SPT, median age 11 months (range 3-24 months), with 10 males and 12 females. The control group had 23 healthy children, median age 12 months (range 3-34 months), with 13 males and 10 females. Median IgE levels were significantly increased in AD patients (SPT-Pos + SPT-Neg groups) compared to the control group [210 (range 50-843) IU/mL vs. 140 (range 3-450) IU/mL, respectively; ($P=0.015$)]. However, no significant difference in IgE levels was found between the SPT-Pos and SPT-Neg groups [230 (range 50-710) IU/mL vs. 204 (range 58-843) IU/mL, respectively; ($P=0.86$)]. Median eosinophil counts were also significantly

Table 1. Patient characteristics and median values of age, total IgE, eosinophil count, and LXB4

Characteristics	SPT-Pos group n=21	SPT-Neg group n=22	Control group n=23
Gender			
Female	12	12	10
Male	9	10	13
Median age (range) months	8 (3-28)	11 (3-24)	12 (3-24)
Median total IgE (range), IU/mL	230 (50-710)	204 (58-843)	140 (3-450)
Median eosinophil (range), n/mL	420 (120-1030)	280 (60-710)	176 (4-450)
Median LXB4 (range), pg/mL	1.55 (0.96-3.49)	1.11 (0.46-1.80)	0.80 (0.28-1.99)

higher in the AD groups than the control group [370 (range 60-130) /mL) *vs.* 176 (range 4-450) / mL), respectively; (P=0.001)]. As expected, the median eosinophil count in the SPT-Pos group was higher than in the SPT-Neg group [420 (range 120-1030)/mL *vs.* 280 (range 60-710)/mL, respectively; (P=0.021)]. Furthermore, median serum LXB4 level was significantly higher in the AD patients (SPT-Pos + SPT-Neg groups) than in the control group [1.43 (range 0.46-3.49) pg/mL *vs.* 0.80 (range 0.28-1.99) pg/mL, respectively; (P=0.004)]. In binary comparisons among the groups, serum LXB4 level was higher in the SPT-Pos group than in the SPT-Neg group [1.55 (range 0.96-3.49) pg/mL *vs.* 1.11 (range 0.46–1.80) pg/mL, respectively; (P=0.002)]. Also, the LXB4 level in the SPT-Pos group was significantly higher than in the control group [1.55 (range 0.96-3.49) pg/mL *vs.* 0.80 (range 0.28-1.99) pg/mL, respectively; (P=0.0001)]. No significant difference in the median LXB4 was observed between SPT-Neg and control groups (P=0.26) Also, a positive correlation was observed between LXB4 levels and eosinophil counts in all groups (r=0.316, P=0.010) (**Figures 1** and **2**).

Discussion

Atopic dermatitis, allergic rhinitis, and asthma are common allergic conditions with high morbidity. To our knowledge, no one has investigated a possible role of serum LXB4 in the pathogenesis of AD in children. In our study, serum LXB4 levels were significantly higher in AD patients than in controls, especially in the SPT-Pos group. Also, serum LXB4 levels were positively correlated with eosinophil counts. Chronic inflammation is one of the most

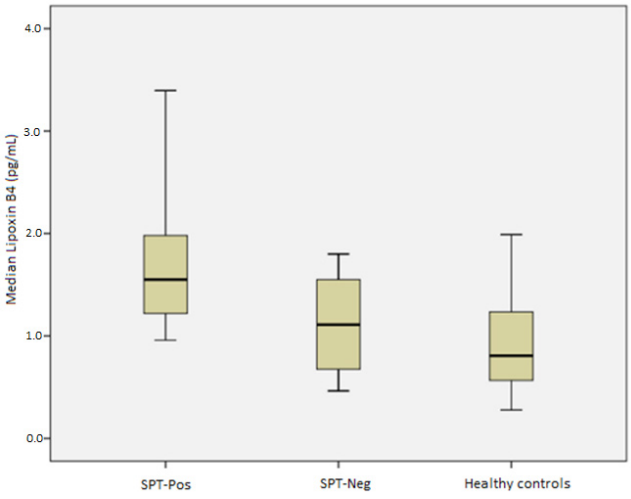

Figure 1. Increased LXB4 in children with atopic dermatitis

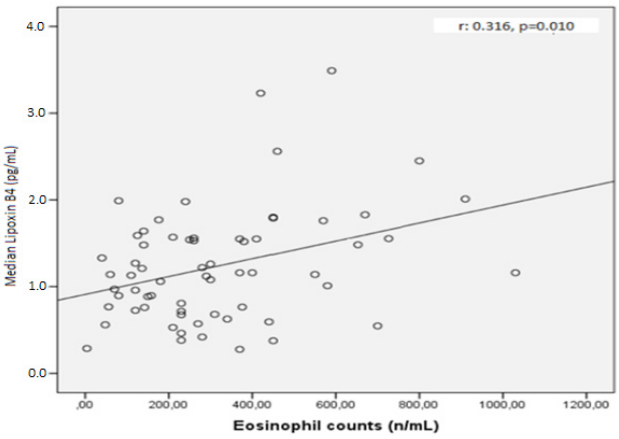

Figure 2. Positive correlation between LXB4 levels and eosinophil counts

important characteristics of patients with AD. At sites of inflammation, LXs are synthesized from membrane phospholipids and have anti-inflammatory effects.[9] The LXA4 blocks histamine release from mast cells and decreases the degranulation of azurophilic granules from neutrophils.[10-11] The LXB4 is also a product of endogenous arachidonic acid metabolism which has anti-inflammatory effects.[12] In a recently reported animal study on allergic airway diseases, LXB4 reduced leukocyte infiltration and mucus secretion in the nasal mucosa. Also, LXB4 decreased degranulation of mast cells and eosinophils, in addition to reducing serum specific IgE levels, eosinophil chemotaxis, and cytokine release from mast cells following IgE-mediated activation. Consequently, the authors concluded that LXB4 regulates allergic inflammation as an anti-inflammatory mediator.[4] In our study, serum LXB4 levels were higher in AD patients than in controls, which suggests a potential role of LXB4 in the pathogenesis of AD. In allergic diseases, different results have been reported with regards to LXs, especially LXA4. A previous study reported that serum LXA4 levels were lower in wheezy infants than in controls.[13] In contrast, increased LXA4 levels were reported in exhaled breath condensate in asthmatic children, which was in parallel with our results.[14] Also, higher LXA4 concentrations were found in asthmatic patients than in control subjects in induced sputum.[9] In an animal model of asthma and allergic rhinitis, LXB4 had an anti-inflammatory effect on allergic mucosal inflammation.[4] Moreover, in a previously published human study, local LXA4 application improved the severity eczema scale score, eczema area and severity index, and dermatologic quality of life index for infants, with effects similar to local corticosteroid (mometasone) treatment of children with AD. In that study, AD could be temporarily controlled and effectively treated with an LXA4 analog.[15]

In conclusion, the higher levels of LXB4 seem to reflect response to chronic inflammatory processes in patients with AD. There is no curative therapy for chronic allergic inflammation in patients with AD. For this purpose, new therapeutic approaches are needed to treat allergic diseases. The LXB4 and related compounds may provide novel therapeutic approaches for the topical treatment of human skin diseases such as AD.

Conflict of interest

None declared.

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Original Article

Growth and developmental delay risk factors among under-five children in an inner-city slum area

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Abstract

Background Growth and developmental delays are common among children under the age of five years (under-five children), especially in slum areas. Early detection and intervention may give better prognoses.

Objective To detect growth and developmental delays and related risk factors among under-five children living in an inner-city slum area of the Indonesian capital.

Methods This cross-sectional study was conducted from October to November 2018 in Tanah Tinggi, Johar Baru District, an inner-city slum area in Central Jakarta. Subjects were healthy children aged 3-60 months. Socioeconomic profile was obtained through questionnaires, anthropometric data through measurements, and developmental status through the *Kuesioner Pra Skrining Perkembangan* (KPSP) instrument. Development was considered to be delayed for KPSP scores <9. Data were analyzed using Chi-square test.

Results Of 211 subjects, prevalence of underweight, stunting, and wasting were 35.1%, 28.0%, and 20.9%, respectively, meanwhile low maternal education, and low family income were 57.9% and 75%. The prevalence of developmental delay was 10%, while suspected developmental delay was 26.1%. The prevalence increased from age 21 months and peaked at 36 months. Associated risk factors were low maternal education, low family income, underweight weight-for-age, stunted height-for-age, and microcephalic head circumference-for-age.

Conclusion Low education and low income were significant risk factors for growth and developmental delay. [Paediatr Indones. 2019;59:276-83; doi: http://dx.doi.org/10.14238/pi59.5.2019.276-83].

Keywords: *development; growth; slum area*

Growth and development from conception to adolescence is characteristic of the childhood phase.[1] Growth can be monitored through increments of weight, height, and head circumference, while development is marked by increases in individual abilities, such as gross and fine motor skills, hearing, vision, communication, social-emotion, independence, intelligence, and moral.[2] Rapid growth and development occur in the first five years of life, therefore, close monitoring and early detection of delays during this critical period is crucial.[1,3]

Close monitoring of children's development can be done using questionnaires. A practical and widely-used questionnaire in Indonesia is the *Kuesioner Pra Skrining Perkembangan* (KPSP). This KPSP is the Indonesian version of the *Prescreening Developmental Questionnaire* (PDQ), modified by the Republic of Indonesia Ministry of Health in 1996 and revised in 2005.[1] With sensitivity of 60% and specificity of 92%,[3] KPSP is recommended for use in primary healthcare services as an early detection method for

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developmental problems in children.[1] Developmental delays are common, especially in children living in slum areas, with prevalences of 12-16% in America and 13-18% in Indonesia. However, most cases of developmental delay remain underdiagnosed and untreated, despite the established premise that children with developmental delays have better prognoses if the problem can be detected and treated earlier.[3] Developmental screening of children aged 6-12 months using the KPSP in Bandung, West Java, Indonesia, showed prevalences of suspected developmental delay and actual delay of 13.6% and 0.4%, respectively. Early intervention for children with suspected developmental delay significantly reduced the number of cases.[4] Despite the known benefits, only 2-3% of all children receive public early intervention services by the age of 3 years.[5] Therefore, identifying risk factors for developmental delay in children may provide better understanding and more effective approaches for early detection as well as early intervention in certain populations.

The aim of this study was to detect growth and developmental delays and their related risk factors among under-five children living in an inner-city slum area in the Indonesian capital.

Methods

A cross-sectional study was conducted in Tanah Tinggi, Johar Baru District, an inner-city slum area in Central Jakarta. This area was a densely populated slum area of low socioeconomic level. Children aged 3-60 months living in this area were included in this study, and selected by stratified random sampling. Data collection was conducted in 3 days: 19 October 2018, 26 October 2018, and 9 November 2018. Patients with Down syndrome, cerebral palsy, or hydrocephalus were excluded from the study. Using the sample size formula for cross-sectional studies,[6] the expected proportion of developmental delay in Indonesia was 13%. Hence, the minimum required sample size calculated for this study was 174 subjects.

This study was approved by the Ethics Committee of the Universitas Indonesia Medical School. All the participants' parents or caregivers provided written informed consent prior to this study.

Subjects' data were obtained through interviews, anthropometric measurements, and KPSP examinations conducted by doctors from the Department of Child Health, Universitas Indonesia Medical School. Interviews of mothers or caregivers were done using a questionnaire that consisted of parental and child identities, primary caregiver, total number of children in family, maternal education level, maternal working status, and family income. Total number of children in the family was classified as either 1-2 or more than 2. Maternal education level was classified as either low (below junior high school) or high (senior high school and above). Family income was classified based on the DKI Jakarta province regional monthly minimum wage in 2018 as either low (below Rp 3,600,000) or high (Rp 3,600,000 and above).

Weight, height, and head circumference measurements were done to obtain anthropometric data of all subjects. Body weight was measured using a calibrated scale (*Seca*®), with accuracy to 1 gram, while subject was wearing minimal clothing. Body length was measured in a recumbent position using a length board (*Seca*®), with accuracy to 1 mm for children <2 years old, and body height was measured in a standing position using a height board (*Seca*®), with accuracy to 1 mm, for children ≥2 years old. The measurement for recumbent length was done while the child was lying on his back with head against the fixed headboard, compressing the hair, eyes looking straight up, legs straight, and soles of the feet flat against the footboard. The measurement of standing height was done while the child was standing on the baseboard with feet slightly apart, backs of the head, shoulder, buttocks, calves, and heels touching the vertical board, and a horizontal line from ear canal to the lower border of the eye socket running parallel to the baseboard.[7] The measurement of head circumference was done using a measuring tape (*Seca*®) around the broadest part of the forehead above the eyebrows, ears, and the most prominent part of the back of the head, with an accuracy of 1 mm.[8] The results were plotted on the *2005 World Health Organization* (WHO) growth curve to determine nutritional status using weight-for-length (WFL), weight-for-age (WFA), and length/height-for-age (LFA/HFA), then interpreted based on WHO growth indicators.[9] The WFA was categorized as normal weight (-2SD ≤ *z*-score <+1SD), underweight (-3SD ≤ *z*-score <-2SD), severely underweight (*z*-score <-3SD), or

having a risk of overweight (z-score ≥+1SD). The LFA/HFA was categorized as normal height (-2SD ≤ z-score <+3SD), stunted (-3SD ≤ z-score <-2SD), or severely stunted (z-score <-3SD). The WFL was categorized as good nutritional status (-2SD ≤ z-score <+2SD), wasted (-3SD ≤ z-score <-2SD), severely wasted (z-score <-3SD), or overweight (z-score ≥+2SD). Head circumference-for-age (HCA) was plotted on a Nellhaus curve,[10] then interpreted as normocephalic (-2SD ≤ HC <+2SD), microcephalic (HC <-2SD), or macrocephalic (HC >2SD). Children with any anthropometric problems (underweight, stunted, wasted, or microcephalic) were categorized as having a growth disturbance.

Developmental screening was conducted using the KPSP. This questionnaire was used as a preliminary screening tool for children aged 3 months to 6 years, and consists of 10 questions about ability based on the child's age group. The questions were answered by parents or caregivers with a 'yes' or 'no.' If the total number of yeses was 6 or below, developmental delay was suspected and the child was referred for further comprehensive evaluation. If the total number of yeses was 7-8, the result was inconclusive and re-examination was done within 1-2 weeks. If the total number of yeses was 9-10, the child was considered to have normal development, but routine KPSP examination in the next age grouping should be performed. In our study, KPSP results were categorized as normal (total score ≥9) or abnormal (score <9).

Data are presented in tables with frequency and percentage for each category. Differences in proportions of anthropometric results based on socioeconomic profiles and KPSP results were analyzed using Chi-square test with *SPSS version 20.0* software. Results with P values <0.05 were considered to be statistically significant.

Results

We examined 290 children during the study period (October 2018 until November 2018), of whom 5 children were excluded, 52 dropped out due to incomplete data, and 22 dropped out due to uncooperativeness during examination (refused, slept, or cried). Thus, 211 children aged 3-60 months were included in this study. The median age of subjects was 30 (range 3-59) months with a nearly proportional ratio of males and females. Subjects' characteristics are presented in **Table 1**.

The KPSP results were normal in 135 children (64%), inconclusive in 55 children (26.1%), and suspected developmental delay referral in 21 children (10%). Growth disturbance was reflected by the prevalence of underweight (WFA <-2SD), stunting (LFA/HFA <-2SD), and wasting (WFL <-2 SD), which were 35.1%, 28.0%, and 20.9%, respectively. The prevalence of microcephaly in this study was 17.1%. Of 21 subjects with suspected developmental delay, 42.9% were underweight and severely underweight, 38.1% were stunted and severely stunted, 23.8%

Table 1. Subjects' characteristics based on KPSP results

Characteristics, n(%)	Normal KPSP (n=135)	Abnormal KPSP (n=76)
Gender		
Male	64 (47.4)	40 (52.6)
Female	71 (52.6)	36 (47.4)
Total number of children in family		
1–2	98 (72.6)	52 (68.4)
>2	37 (27.4)	24 (31.6)
Maternal education		
High	85 (63.0)	32 (42.1)
Low	50 (37.0)	44 (57.9)
Maternal working status		
Working	17 (12.6)	12 (15.8)
Non-working	118 (87.4)	64 (84.2)
Family income		
High	53 (39.3)	19 (25.0)
Low	82 (60.7)	57 (75.0)
Weight-for-age (WFA)		
Severely underweight	5 (3.7)	11 (14.5)
Underweight	32 (23.7)	26 (34.2)
Normal weight	91 (67.4)	35 (46.1)
Risk of overweight	7 (5.2)	4 (5.3)
Length/height-for-age (LFA/HFA)		
Severely stunted	9 (6.7)	3 (3.9)
Stunted	22 (16.3)	25 (32.9)
Normal height	104 (77.0)	48 (63.2)
Weight-for-length (WFL)		
Severely wasted	1 (0.7)	5 (6.6)
Wasted	23 (17.0)	15 (19.7)
Good nutritional status	106 (78.5)	52 (68.4)
Overweight	5 (3.7)	4 (5.3)
Head circumference for age (HCA)		
Microcephalic	15 (11.1)	21 (27.6)
Normocephalic	120 (88.9)	55 (72.4)

were wasted and severely wasted, and 42.9% were microcephalic. Associations between socioeconomic factors and growth disturbances are shown in **Table 2**. Significantly more children with mothers of low education were underweight (WFA) (P=0.009) and wasted (WFL) (P=0.004) than children with mothers of high education. In addition, significantly more children with low family income were underweight (WFA) (P=0.027) than those with high family income.

Associations between developmental delay and socioeconomic factors as well as anthropometric results are shown in **Table 3**. Significantly more children with abnormal KPSP scores had mothers with low education, low family income, underweight WFA status, stunted HFA status, and microcephalic HCA status than children with normal KPSP scores.

The age distribution of subjects with abnormal KPSP scores is presented in **Figure 1**. Most subjects with developmental delay were in the 36-month age group, while most subjects with inconclusive results were in 42-month age group. All subjects with KPSP

Table 2. Associations between socioeconomic factors and growth disturbances

Variables	WFA*	LFA/HFA**	WFL***	HCA****
Total number of children in family				
OR (95% CI)	0.96 (0.51-1.80)	1.39 (0.73-2.65)	1.36 (0.67-2.77)	0.66 (0.2-1.54)
P value	0.900	0.319	0.394	0.331
Maternal education				
OR (95% CI)	2.15 (1.21-3.81)	1.72 (0.94-3.15)	2.68 (1.35-5.33)	1.96 (0.95-4.05)
P value	0.009†	0.078	0.004†	0.068
Maternal working status				
OR (95% CI)	1.15 (0.51-2.59)	1.43 (0.62-3.29)	0.99 (0.38-2.60)	1.68 (0.66-4.29)
P value	0.728	0.400	0.981	0.290
Family income				
OR (95% CI)	2.02 (1.08-3.81)	1.76 (0.90-3.45)	1.73 (0.81-3.66)	1.43 (0.65–3.15)
P value	0.027†	0.097	0.151	0.378

* Categories used for analysis were underweight (underweight and severely underweight) and normal weight (normal and risk of overweight).
** Categories used for analysis were stunted (stunted and severely stunted) and normal height (normal).
*** Categories used for analysis were wasted (wasted and severely wasted) and good nutritional status (good and overweight).
**** Categories used for analysis were microcephalic and normocephalic.
†P< 0.05.

Table 3. Associations between developmental delay (abnormal KPSP scores) and socioeconomic factors as well as anthropometric results

Variables	OR (95%CI)	P value
Gender	1.23 (0.70-2.16)	0.466
Total number of children in family	1.22 (0.66-2.26)	0.521
Maternal education	2.34 (1.32-4.15)	0.003†
Maternal working status	1.30 (0.59-2.89)	0.517
Family income	1.94 (1.04-3.62)	0.036†
WFA*	2.51 (1.40-4.52)	0.002†
LFA/HFA**	1.96 (1.06-3.62)	0.031†
WFL***	1.65 (0.84-3.24)	0.143
HCA****	3.06 (1.46-6.37)	0.002†

* Categories used for analysis were underweight (underweight and severely underweight) and normal weight (normal and risk of overweight).
** Categories used for analysis were stunted (stunted and severely stunted) and normal height (normal).
*** Categories used for analysis were wasted (wasted and severely wasted) and good nutritional status (good and overweight).
**** Categories used for analysis were microcephalic and normocephalic.
† P< 0.05.

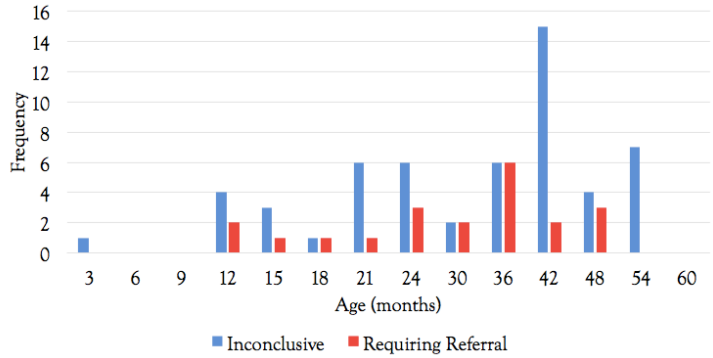

Figure 1. Age distribution of subjects with abnormal KPSP result

results requiring referral had global developmental delay (GDD), which was delay in 2 or more domains of development. Sixteen children were delayed in gross motor skills, 19 children in fine motor skills, 14 children in language skills, and 15 children in personal-social skills. One mother (primary caregiver) of a child with GDD suffered hearing loss and a speech disorder.

Discussion

Growth disturbances among under-five children living in inner-city slum areas are reflected in the prevalences of underweight (WFA <-2SD) and wasted (WFL <-2SD), which were higher in our study than in the national data from the Indonesian Basic Health Research Report 2018 (underweight: 35.1% *vs.* 10.2%, respectively; wasted: 20.9% *vs.* 17.7%, respectively).[11] However, the prevalence of stunted (LFA/HFA <-2SD) in our study population was lower compared to the national data (28.0% vs. 30.8%, respectively), yet higher than the local DKI Jakarta province prevalence, which was 17.7%.[11] Growth problems in under-five children living in urban slum areas can be caused by inappropriate feeding practices, diseases occurring due to poor sanitation, inappropriate parenting, and lack of access and coordination of public health services.[12]

In our study, low maternal educational level was associated with weight-for-length (wasted). This finding was in agreement with a study by Makoka,[13] which showed that children's nutritional status increased with maternal education. In addition, another Indonesian study conducted in children aged 2 to 4.9 years reported that maternal education to middle school and below was significantly associated with nutritional status of a child. The study finds that maternal education to middle school and below were associated with weight-for-age Z score <-2 (underweight) and height-for-age Z score <-2 (stunted).[14] We noted that maternal education to senior high school was significantly associated with children's improved nutritional status. Mothers with higher education are better placed to receive information about childhood nutrition and are more responsive in facing acute conditions, such as fever and diarrhea,[13] such that weight loss may be prevented. Therefore, one of the strategies for solving nutritional problems in children is strengthening the education sector, especially for girls, by completing their senior high school education, as recommended by the national, 12-year, compulsory education program.[15]

A study reported that in children aged 1-3 years old in Sidoarjo, East of Java, weight-for-age was a significantly related variable to family income, but not to maternal working status.[16] Sufficient family income provides the means to better meeting the nutritional requirements of children.

There was no significant difference in socioeconomic factors between stunting and normal height in our study. This finding might have been due to the limited sample size. In addition, several factors associated with height-for-age were not studied, such as history of intrauterine growth restriction, premature birth, poor sanitation, infectious disease, as well as energy and protein intake.[17,18] These factors may play

important roles in linear growth in children under-five living in urban slum areas.

There was also no significant difference in socioeconomic factors between normocephalic and microcephalic children under-five. Our finding was in agreement with a study conducted in Kenyan children. Abubakar *et al.*[19] found that wealth index and maternal education had no significant associations with head circumference. A relatively larger contribution of genetic factors might explain this finding. Head circumference is less susceptible to socioeconomic factors compared to other anthropometric measurements. However, our results differed from that of Bouthoorn *et al.*[20] who found that children born to highly-educated mothers had significantly larger head circumference compared to children born to mothers with low and mid-low educational levels. This finding may have been due to differing methods of interpretation of microcephaly as well as differing classifications of maternal education levels.

The prevalence of developmental delay in Tanah Tinggi, Johar Baru District, Central Jakarta was 10%. Previous studies using the same instrument (KPSP) in children under-five showed similar numbers of 10% in Malang, East Java, and 8% in Bantul, Yogyakarta.[3,21]

Five variables had statistically significant associations with developmental delay: low maternal education, low family income, underweight weight-for-age, stunted height-for-age, and microcephalic head circumference-for-age. Similarly, a previous study in Bantul, Yogyakarta found that low maternal education, low socioeconomic status, and maternal working outside the home were risk factors for developmental delay in children under-five.[21] Demirci *et al.*[22] concluded that developmental delay was related to advanced maternal age, low maternal education, low socioeconomic status, and consanguinity.

Maternal working status, total number of children in the family, and nutritional status according to weight-for-height were not significantly related to developmental delay in our subjects. These findings might have been caused by another important unevaluated factor, stimulation in all area of child development. Non-working mothers with low education may not provide enough stimulation to their children due to lack of knowledge about the importance of stimulation for childhood development.[23]

There were 76 children with abnormal KPSP results in our study, 55 children with (inconclusive and 21 children with suspected developmental delay. Children with inconclusive should undergo re-examination within 1 week, while children with suspected developmental delay should be referred for further examination. Delays were distributed in four aspects of development, yet the highest number of children were delayed in fine motor skills. In contrast, Fadlyana *et al.*[24] in Bandung showed that most delays occurred in vocalization and language comprehension. Twenty-one children with confirmed developmental delay had global developmental delay. However, the mother of one such child had a hearing loss and speech disorder. As the primary caregiver, her child's delay may have been caused by lack of stimulation. Based on age groups, the incidence of developmental delay increased from 21 months of age and peaked at 36 months. This result suggests that early detection and intervention for development should be started before 21 months of age, or within first the 1000 days of life to achieve optimum results.[25]

Malnutrition and inadequate stimulation are the main risk factors for disturbances in cognitive, motor, social behavior, school behavior, and psychomotor development. Nutrition and stimulation play important roles in brain development in the first five years of life.[26,27] In our study, 21 children with confirmed developmental delay had nutritional problems reflected by anthropometric results, 42.9% underweight and severely underweight, 38.1% stunted, 23.8% wasted and severely wasted, and 42.9% microcephalic. A cross-sectional study involving children under-five in Nigeria found that weight-for-age had significant associations with the hearing and language domain (P=0.036) and the interactive social domain (P=0.001). Underweight children were three times as likely to have delays in hearing and language skills and five times as likely to have delays in interactive social skills.[26] A systematic review and meta-analysis by Sudfeld *et al.*[28] concluded that linear growth as reflected by height-for-age was associated with cognitive and motor development in children in developing countries. Nutritional status was a predictor in hearing, language, and social interaction abilities. Malnutrition results in delayed auditory system maturation, which affects both the

central and peripheral auditory systems. Children with malnutrition have difficulty understanding information, causing them to be apathetic and indolent in exploring their surroundings, and resulting in delayed social interaction skills. Such conditions may impact children's academic achievement as well as work-related skills in the future.[26]

In our study, 42.9% of children with developmental delay had microcephaly, 50% children with developmental delay had chronic malnutrition as reflected by wasting and stunting. In microcephalic children, 0.1% may be asymptomatic, while 15-20% manifest developmental delay.[29] Microcephaly can be caused by genetic disorders as part of a syndrome, teratogens, infection, metabolic disorders, as well as prenatal, perinatal, or post-natal problems.[30] Microcephaly was associated with lower developmental quotient (DQ) and higher morbidities (epilepsy, hearing disorders, and visual impairments).[28]

Our study had several limitations. The KPSP was evaluated in an open space near the anthropometric measurement location with a little bit noisy and distractful environment, which may cause inconvenience to the children. This situation resulted in a high number of dropouts due to uncooperativeness during the examination. We suggest that further community study on child development be done in a comfortable and quiet room in order to provide a conducive environment for examination. In addition, we did not evaluate other important factors contributing to development such as parental stimulation, home environment, and comorbidities. Further study should include evaluation of such factors.

In conclusion, prevalence of underweight and wasting in children under-five in an inner-city slum area are higher than their corresponding national data, while the prevalence of stunting is higher than provincial data. Based on KPSP results, the prevalence of developmental delay in children under-five in Tanah Tinggi, Central Jakarta is 10%, and suspected developmental delay is 26.1%. Risk factors for growth disturbances are low maternal education and low family income, while risk factors for developmental delay are low maternal education, low family income, underweight weight-for-age, stunted height-for-age, and microcephalic head circumference-for age. All children with developmental delay or malnutrition were referred to the Tanah Tinggi Primary Health Center for further evaluation. Growth and development in children should be monitored closely and routinely so the intervention can be done as soon as possible, especially in the first 1000 days of life.

Conflict of Interest

None declared.

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Case Report

Complications of drowning: a case report

Felicia Anita Wijaya, I Gde Doddy Kurnia Indrawan

Unintentional drowning is the sixth most common cause of accidental death, accounting for 4,086 deaths (1.4 per 100,000) in the United States in 2007.[1] In children, drowning is the second leading cause of injury-related death, and those aged 1-3 years have the highest rate of drowning.[2] More than 1,400 pediatric drownings were reported in the United States in 2008.[3] Many drowning deaths are due to lack of supervision in the bathtub, unprotected access to a pool, or lack of swimming skills.[3] For every death by drowning, six children are hospitalized for drowning, and up to 10% of survivors experience severe brain damage.[2] **[Paediatr Indones. 2019;59:284-8; doi: http://dx.doi.org/10.14238/pi59.5.2019.284-8**].

Keywords: *bronchopneumonia; case report; drowning; mechanical ventilation; pulmonary edema*

Drowning is the process of experiencing respiratory impairment from submersion/immersion in liquid. In 2010, drowning was the leading cause of injury death for children 1 to 4 years of age and the second leading cause of injury death for children 1 to 18 years of age.[4] Submersion injuries occur in domestic settings such as swimming pools, hot tubs, bathtubs, large buckets, rainwater tanks, and in all forms of natural bodies of water. Age, gender, and race affect the incidence of drowning. According to *WHO*, in 2015, an estimated 360.000 people died from drowning, making drowning a major public health problem worldwide.[5] Toddlers and older teenagers are at greatest risk of death by drowning, with annual incidences of 2.46 and 1.47 per 100,000, respectively.[1,6] Boys account for almost 80% of victims older than 1 year. Black males between 15 and 19 years of age have the highest annual incidence of drowning mortality (3.92 per 100,000), and black children between the ages of 5 and 14 years drown at nearly three times the rate of white children of the same age.[1] The risk of death by drowning within the American Indian population is twice as high as it is for the white population.1 Other risk factors include a history of seizure, alcohol use, and lack of supervision.[2,7]

The drowning process begins with respiratory impairment as the person's airway goes below the surface of the liquid (submersion) or water splashes over the face (immersion).[8] Unexpected submersion triggers breath-holding, panic, and struggling to surface. Air hunger and hypoxia will develop, and the victim begins to swallow water.1 Breath-holding is overcome by involuntary gasps resulting in aspiration, leading to laryngospasms.[1,7] The progressive decrease in arterial blood oxygen saturation (SaO2) soon causes the victim to lose consciousness due to hypoxia.[7]

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At the same time, the cardiovascular response progressively decreases cardiac output and oxygen delivery to other organs. By 3-4 minutes, myocardial hypoxia leads to abrupt circulatory failure.[1] Profound hypoxia as well as metabolic and respiratory acidosis ensue, leading to cardiovascular collapse, neuronal injury, and ultimately death.[1]

Organs such as the brain, lungs, and kidneys are mainly affected by drowning accidents. However, the greatest permanent harm in drowning accidents is to the brain, which has negligible metabolic substrate reserves to subsist upon in the absence of continuous delivery of oxygenated blood. Much of the literature on near-drowning has concentrated on the respiratory effects of aspiration and on the management of both early and late respiratory complications such as aspiration pneumonia and adult respiratory distress syndrome.[8]

Many factors may influence the pathophysiologic sequence of events in submersion injury and affect the chance of survival, including age, water temperature, time of submersion, duration and degree of hypothermia, diving reflex, presence of aspiration (and pulmonary parenchymal injury), and effectiveness of resuscitative efforts.[1,3] Poor outcome risks include victim age younger than 3 years, submersion for longer than 5 to 10 minutes, and initiation of cardiopulmonary resuscitation (CPR) more than 10 minutes after rescue. But specifically, the outcome of drowning is determined by the success of immediate resuscitation efforts and the severity of the hypoxic-ischemic injury to the brain. Hypoxia, which is usually dependent on submersion time, is the most important factor related to outcome and subsequent quality of life in drowning victims.[1] Patients who have regained consciousness on arrival to the hospital will likely survive with intact neurologic function.[4]

The Case

A girl aged 1 year and 8 months was admitted to the emergency room of Wangaya Regional Hospital, Denpasar, Bali on September 27, 2018 due to loss of consciousness after submersion. The child's father had been bathing her and was called away by the sister. He left the child alone in the bathtub, never thinking about the possibilty of drowning. After approximately 5 minutes, the father returned to the bathroom and found his child unconscious, not moving at all, and cyanotic. Immediately, the father took his child to the emergency room of Wangaya Regional Hospital, about 5 minutes from their house. The vital signs showed that she was unconscious, not breathing, pulseless, and hypothermic. The Glasgow coma scale was 1 for eye response, 1 for motor response, and 1 for verbal response. The patient was immediately resuscitated with 1 cycle of cardiopulmonary resuscitation (CPR), had an oropharyngeal tube inserted, and given Ringer's lactate infusion. Suctioning of the oropharnyx was done and brown fluid was extracted. The patient cried for 20 seconds and vomited a few times, but she was apathetic. The pulse began to be palpable at 128 beats per minute, respiratory rate was 34 times per minute, body temperature was 35.8oC, and oxygen saturation was 89%. The patient received non-rebreathing mask treatment of 10 liters per minute and was wrapped in a blanket.

The patient had no previous history of drowning or other disease, nor did the family. She was born vigorous, during the 38th week of gestation, by normal delivery, with birth weight of 2,700 grams, and body length of 50 cm. Her growth and development was normal. She could lift up her head at 3 months, sit well at 7 months, stand up at 11 months, and walk properly at 12 months. She could speak two-syllable words such as "ma-ma" and "da-da" beginning at the age of 9 months. Her basic immunizations were complete.

Her nutrition history included breast milk from birth to the date of the event. She started eating complementary food at 6 months of age and solid food at 12 months of age. Her body weight was 12 kg, with weight/age score of 0-2 standard deviation (SD). Her body height was 88 cm with height/age score of 0-2 SD. Her weight/height score was 0 SD, indicating that her nutritional status was normal.

Physical examination was held after the resuscitation. Eyes examination revealed isocorous pupils and positive reflex in both corneas. We found no abnormalities of the heart or abdomen. There was no asymmetrical chest movement. There was a decrease in both vesicular lung sounds and rales in both lungs. The extremities were cyanotic, but improved after resuscitation, with capillary refill time of less than three seconds. There was no sign of

physical injury. Chest x-rays showed patchy shadowing with air bronchograms in both lung fields (**Figure 1**). Laboratory tests revealed increased leukocyte, plaletet count, lymphocyte, and random glucose level (**Table 1**).

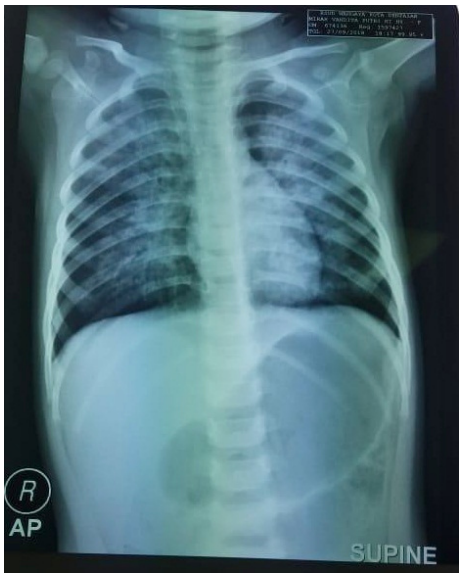

Figure 1. Chest x-ray showed patchy shadowing with air bronchograms in both lung fields

The patient was transferred to the PICU, where she was intubated, and received mechanical ventilation, as well as Ringer's lactate infusion at 15 drops per minute. She was given ceftriaxone at 600 mg every 12 hours as an antibiotic, dexamethasone at 2 mg every 8 hours as a corticosteroid, ranitidine at 15 mg every 8 hours as a histamine type-2 receptor antagonist, furosemide at 10 mg every 12 hours as a diuretic, and budesonide as a corticosteroid nebulizer alternating with salbutamol as a bronchodilator nebulizer, every 6 hours.

After 24 hours the patient was fully conscious and extubated. She had a cough, but no other complaints. Gradually the patient's condition improved to normal. The patient was discharged from the hospital on the fourth day after admission.

Discussion

Most drowning deaths (71%) in children occur in the bathtub.[7] Infant tub seats or rings may exacerbate the risk by giving caregivers a false sense of security that the child is safe in the tub.[7] In our case, the child was left alone in the bathtub by the father.

In the emergency room, the patient immediately received resuscitation after assessing that she was unconscious, apneic, and pulseless. Resuscitation

Table 1. Laboratory test results

Hematology	Results		Reference range
	September 27, 2018 (16.37 UTC+08.00)	September 28, 2018 (21.00 UTC+08.00)	
Hemoglobin	11.1 g/dL	-	12.0-16.0 g/dL
Erythrocyte (RBC)	4.22 x 106/µL	-	4.00-5.30 x 106/µL
Leukocyte (WBC)	21.09 x 103/µL	-	5.0-13.0 x 103/µL
Hematocrit	35.3 %	-	35.0-45.0 %
Platelet count	499 x 103/µL	-	150-400 x 103/µL
MCV	83.6 fL	-	75.0-91.0 fL
MCH	26.3 pg	-	25.0-33.0 pg
MCHC	31.4 g/L	-	31.0-37.0 g/L
Neutrophil	10.2 %	-	32-52 %
Lymphocyte	85.9 %	-	30-60 %
Monocyte	3.4 %	-	2-8 %
Random glucose	249 mg/dL	160 mg/dL	80-200 mg/dL

included the basic ABCs (airway, breathing, and circulation), which were head tilt chin lift, oropharyngeal tube, cardiopulmonary resuscitation, and Ringer's lactate infusion, simultaneously.[1,4,7] Initial resuscitation of drowning victims must focus on rapidly restoring oxygenation, ventilation, and adequate circulation.[7]

Vascular access should be established as quickly as possible for the administration of fluids or pressors. Intraosseous catheter placement is a potentially lifesaving vascular access technique that is usually associated with multiple attempts to establish intravenous access in critically ill children. Epinephrine is generally the initial drug of choice in victims with bradyasystolic cardiopulmonary arrest.[7] But for this case, it was not necessary to give epinephrine because after intial resuscitation, the patient's vital sign was improved.

Hypothermia in pediatric drowning victims may be observed even after drowning in relatively warm water and in warm climates.[7] The symptoms and severity of hypothermia are categorized based on body temperature. Mild hypothermia is defined as a temperature of 34-36°C (93.2-96.8°F), with intact thermogenic mechanisms (shivering and nonshivering thermogenesis, vasoconstriction) and active movements. With moderate hypothermia [30 to <34°C (86 to <93.2°F)], loss of consciousness leads to water aspiration. At body temperatures <28°C (82.4°F), extreme bradycardia is usually present with decreases in cardiac output, and the propensity for spontaneous ventricular fibrillation or asystole is high. Central respiratory center depression with moderate to severe hypothermia results in hypoventilation and eventual apnea. A deep coma, with fixed and dilated pupils and absence of reflexes at very low body temperatures [<25-29°C (77-84.2°F)], may give the false appearance of death.[7] The patient in this case experienced mild hypothermia with a body temperature of 35.8°C, which was high enough to rewarm her with only a blanket.

Further treatment is based on the patient response to initial resuscitation. Some children begin breathing spontaneously and awaken before arrival at an emergency department. If the episode was significant, these children still require careful observation for pulmonary complications over the subsequent 6 to 12 hours.[1] Children should be monitored in an intensive care unit for advanced life support and management of multiorgan dysfunction.[7] Pulmonary dysfunction often results in hypoxemia. Oxygen supplementation should be implemented to maintain normal oxygen saturations. Mechanical ventilation may be needed in patients with significant pulmonary or neurologic dysfunction.[4] In our case, after resuscitation, the patient was still apathetic with pulse rate 128 times per minute, respiratory rate 34 times per minute, and oxygen saturation 89%. Hence, we decided to perform intubation and mechanical ventilation in the PICU.

Submersion victims swallow a significantly greater volume of water than is aspirated, and gastric distention from positive-pressure ventilation during rescue is common. As a result, 60% of patients vomit after a submersion event.[1,5] Aspiration of gastric contents greatly compounds the degree of pulmonary injury and increases the probability that acute respiratory distress syndrome will ensue.[7] In addition, aspiration of particulate contaminants such as pathogenic organisms, toxic chemicals, and other foreign matter may obstruct the smaller bronchi and bronchioles and greatly increase the risk of infection (both bacterial and fungal in nature).[1,7] Our patient had pulmonary complications of pulmonary edema and bronchopneumonia caused by drowning, as evidenced by her increased respiratory rate and audible rales in both lungs. Chest x-ray revealed patchy shadowing with air bronchograms in both lung fields. Laboratory test showed leukocytosis. Management of our patient included antibiotics to eliminate infection, corticosteroid as an anti-inflammatory agent, histamine type-2 receptor antagonist, diuretic to resolve pulmonary edema, and corticosteroid nebulizer alternating with bronchodilator nebulizer to help resolve symptoms.

Neurologic examination and progression during the first 24-72 hours are the best prognostic factors of long-term central nervous system outcomes. Children who regain consciousness within 48-72 hours, even after prolonged resuscitation, are unlikely to have serious neurologic sequelae. In a small series of comatose victims of non–icy water submersion, all survivors with a good outcome had spontaneous purposeful movements and normal brainstem function within 24 hours; good recovery did not occur in any child with abnormal brainstem function or absence of

purposeful movements at 24 hours.5 Fortunately for this case, the patient regained consciousness within 24 hours with no sequelae. On the fourth day after admission, patient was discharged from the hospital with a good outcome.

Parental education on water safety is important to prevent such incidents. In 2010, the American Academy of Pediatrics Committee on Injury, Violence, and Poison Prevention revised its policy statement on the prevention of drowning to advocate for more anticipatory guidance regarding the appropriate supervision of children, access to swimming lessons, the presence of lifeguards, barriers to swimming pools, and CPR training for adults.[5,6]

Conflict of Interest

None declared.

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Original Article

Diagnostic value of mean platelet volume in neonatal sepsis

Kristopher May Pamudji, I Made Kardana

Abstract

Background Neonatal sepsis is a severe disease with potentially serious impacts if not treated early. However, the symptoms and clinical signs are not specific. Several studies have been conducted to find early infection markers for detection of neonatal sepsis, but without satisfactory results. Mean platelet volume (MPV) is a new marker of infection that has good potential for diagnosing neonatal sepsis.

Objective To assess the diagnostic value of MPV in early detection of neonatal sepsis.

Methods This retrospective study with diagnostic testing was done with data collected from medical records of neonates with neonatal sepsis who were admitted to the Neonatology Department in Sanglah Hospital, Denpasar from December 2018 to March 2019. Mean platelet volume cut-off point was determined using a receiver-operating characteristic (ROC) curve. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of MPV in neonatal sepsis were determined using a 2x2 table.

Results Of 82 subjects, 55 subjects were male (67%). Positive blood culture results were found in 25 subjects (30%). Mean platelet volume with a cut-off point of 7.44 fL had 80% sensitivity, 84.2% specificity, 69% PPV, and 90.6% NPV.

Conclusion Mean platelet volume with a cut-off point of 7.44 fL can be used to diagnose neonatal sepsis with a sensitivity of 80% and specificity of 84.2%. **[Paediatr Indones. 2019;59:289-93; doi: http://dx.doi.org/10.14238/pi59.6.2019.289-93]**.

Keywords: *mean platelet volume; neonatal sepsis; sensitivity; specificity*

Neonatal sepsis is a severe disease with serious impacts if not treated in a timely manner. Neonatal sepsis remains a cause of high perinatal morbidity and mortality, especially in developing countries. The development of medical science in terms of neonatal care has increased the life expectancy of neonates. However, neonatal sepsis still continues to make a significant contribution to neonatal morbidity and mortality.[1]

Neonatal sepsis is a clinical syndrome of systemic disease accompanied by bacteremia that occurs in the first 28 days of life.[2] The incidence of neonatal sepsis varies from 7.1 to 38 per 1,000 live births in Asia, 6.5 to 23 per 1,000 live births in Africa, and 3.5 to 8.9 per 1,000 live births in South America.[3] The national incidence of neonatal sepsis in Indonesia is unknown. However, the incidence of neonatal sepsis in Cipto Mangunkusumo Hospital, Jakarta was 13.7% with a mortality rate of 14%.[4] Sanglah Hospital, Denpasar had a 5% incidence of neonatal sepsis with a mortality rate of 30.4%.[5]

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Neonatal sepsis is a severe systemic disease, but the symptoms and clinical signs are not specific and include temperature instability, tachycardia or bradycardia, hypotension, poor tissue perfusion, metabolic acidosis, apnea, respiratory distress, grunting, cyanosis, lethargy, seizures, feeding intolerance, abdominal distension, jaundice, petechiae, purpura, and bleeding.[6] The gold standard in diagnosing neonatal sepsis is blood culture, however, it requires a long examination time and is not always effective for identifying microorganisms. Hence, a negative blood culture result does not always indicate an absence of microorganisms in the patient. Such a false negative situation can be caused by a small volume blood specimen or administration of antibiotics to the mother before delivery. Some examinations such as white blood cell count, absolute neutrophil count, immature-to-total neutrophil ratio (IT ratio), platelet count, C-reactive protein (CRP), and procalcitonin (PCT) have been used as markers of early infection to help diagnose neonatal sepsis. Yet, they have not been able to provide significant benefits as such.[7]

Several studies have been conducted to find early infection markers of neonatal sepsis with good sensitivity and specificity as well as low cost, so that any health care facility can use it. Complete blood examination is inexpensive and routine in almost all health care facilities. Mean platelet volume (MPV) is an indicator of platelet function that reflects platelet production. Some studies found that MPV was associated with course of the disease, such as in neonatal sepsis, acute pyelonephritis, and gastric cancer.8 As such, MPV may be a new marker of infection with good potential to diagnose neonatal sepsis.8 Hence, we aimed to assess the diagnostic value of MPV in early detection of neonatal sepsis.

Methods

This retrospective study with diagnostic testing design was conducted in the Neonatology Ward at Sanglah Hospital, Denpasar. Data were taken from medical records of patients admitted from December 2018 to March 2019. The inclusion criteria were neonates with suspected neonatal sepsis. The exclusion criteria were patients with incomplete medical record data, immunodeficiency diseases, autoimmune diseases, malignancies, hematological disorders, and major congenital anomalies.

Consecutive sampling was used to collect subjects who met the inclusion criteria until reaching the desired sample size. The minimum required sample size was calculated to be 76 subjects using a diagnostic test formula with sensitivity output, error rate set at 5% with $Z\alpha=1.96$, sensitivity set of 0.75, research precision of 17.5%, and disease prevalence of 0.347.9 This study was approved by the Research Ethics Commission of the Universitas Udayana Medical School/Sanglah Hospital, Denpasar. Operational definitions of variables in this study were as follows: neonatal sepsis was a clinical syndrome of systemic diseases accompanied by bacteremia that occurs in the first month of life, with positive blood culture; mean platelet volume (MPV) was the average platelet size value obtained from complete blood count examination at the time of neonatal sepsis diagnosis. Gestational age was clasified into preterm (<37 weeks) and term (37-42 weeks). Birth weight was divided into low (<2500 grams) and normal (2500-4000 grams). Premature rupture of membranes was rupture of the membranes (amniotic sac) before labor occurs (≥12 hours before delivery). Chorioamnionitis was defined as an infection that occurs in the amniotic membrane (chorion), amniotic fluid (amnion), and placenta. Multiple pregnancy was a pregnancy with two or more fetuses. Asphyxia was a condition where the baby is not immediately crying after birth with an APGAR score in the first minute less than 7.

Data collected in this study were gender, gestational age, birth weight, delivery mode, premature rupture of membranes, chorioamnionitis, multiple pregnancies, asphyxia, blood culture result, and MPV count. Data analysis was carried out using *SPSS software*. Mean platelet volume cut-off point was analyzed by ROC curve. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of MPV in neonatal sepsis were determined by a 2x2 table.

Results

There were 89 neonatal sepsis patients during the study period. Five neonates were excluded because of major congenital anomalies and 2 others were

excluded because of incomplete medical records. Thus, the total sample size was 82 subjects, 55 (67%) of whom were male. Blood culture results were positive in 25 subjects (30%). The characteristics of subjects are shown in **Table 1**.

Table 1. Characteristics of subjects

Characteristics	Blood culture results	
	Positive (n = 25)	Negative (n = 57)
Sex, n		
Male	16	39
Female	9	18
Gestational age, n		
Preterm	16	35
Term	9	22
Birth weight, n		
Low	16	34
Normal	9	23
Delivery mode, n		
Vaginal	12	24
Caesarean section	13	33
Premature rupture of membranes, n	3	9
Yes	22	48
No		
Chorioamnionitis, n		
Yes	0	1
No	25	56
Multiple pregnancy, n		
Yes	2	7
No	23	50
Asphyxia, n		
Yes	2	26
No	23	31
Median MPV (range), fL	10.66 (5.66-16.76)	6.13 (4.22-9.96)

The ROC curve analysis revealed an MPV cut-off point of 7.44 fL for neonatal sepsis, with area under curve (AUC) 0.89 (95%CI 0.81 to 0.98) (**Figure 1**). The MPV cut-off point 7.44 fL had 80% sensitivity, 84.2% specificity, 69% PPV, and 90.6% NPV (**Table 2**).

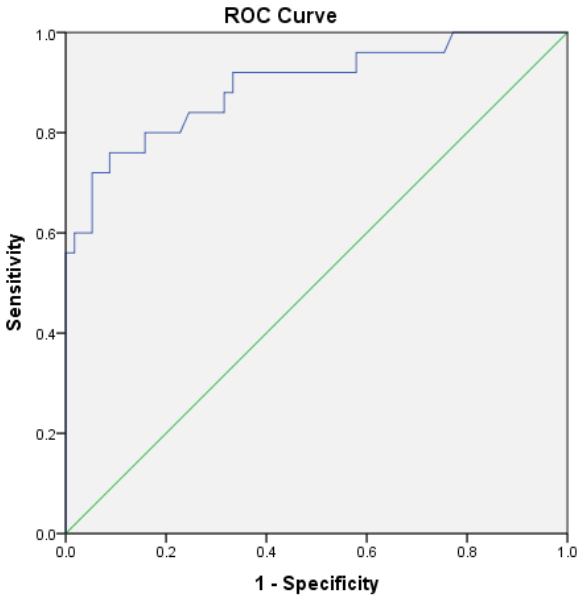

Figure 1. ROC curve of MPV

Discussion

Blood culture is the gold standard for diagnosing neonatal sepsis, but it is insensitive. Maternal antimicrobial treatment and inadequate volumes of blood specimens provided for culture may lead to false negative culture results in infants. Such results can miss up to 75% of cases among those who meet sepsis terminology guidelines.[7] A previous study reported obtaining positive blood culture results in only 40.7% of 344 infants with neonatal sepsis.[10] Similarly, another study obtained positive blood culture results in only 34.7% of 150 infants with neonatal sepsis,[9] and a study obtained positive blood culture results in 41.2% of 102 infants with neonatal sepsis.[11] In our study, blood culture results were positive in only 30% of 82 infants with suspected neonatal sepsis, in accordance with previous studies.

Mean platelet volume is the arithmetic mean volume of the platelets derived from platelet histogram

Table 2. Diagnostic value of MPV in neonatal sepsis

MPV, fL	Blood culture		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative				
≥7.44	20	9	80	84.2	69	90.6
<7.44	5	48				

on an automated Coulter counter. The platelet volume is regulated by cytokine-dependent megakaryocyte ploidy and platelet number.[12] The role of platelets in the inflammatory response is associated with the release of cytokines and chemokines that attract leukocytes and facilitate adhesion to endothelium at the site of damage. Platelets may interact with leukocytes during the inflammatory process by forming platelet-leukocyte aggregates. These bindings are possible through adhesion proteins expressed on the platelets cell surface during activation. Platelets also support leukocytes to eliminate bacterial infections via direct contact, encapsulation of bacteria, and release of reactive oxygen species and platelet microbicidal proteins (PMP).[13]

The mechanism of changing platelet function in sepsis is still unclear. Platelet shape changes from discoid to spherical with pseudopodia during activation. Mean platelet volume reflects the average size of platelets. Young platelets are larger than old platelets. An increased number of young platelets indicates increased platelet production due to overconsumption induced by inflammation. Larger platelets are functionally, metabolically, and enzymatically more active than smaller ones. They contain more intracellular thromboxane A2 and increased expression of procoagulant surface proteins such as P-selectin and glycoprotein IIIa, causing greater prothrombotic potential. Moreover, platelet-neutrophil interactions and platelet-endothelial interactions facilitate a variety of immune activation instances.[14]

Elevated MPV may indicate endothelial damage as well as platelet activation, and is an easily accessible hematological parameter. In a clinical study, thrombocyte consumption and MPV values escalated in acute infections.[15] The studies conducted on neonatal sepsis patients reported that MPV values were high and the increase in the MPV values was significant in terms of prognosis and mortality.[15]

The median MPV in this study was higher in the positive blood culture group compared to the negative blood culture group (10.66 and 6.13 fL, respectively). A study reported that MPV with cut-off point of 9.7 fL was significant and considered to be clinically useful, with 75% sensitivity and 69.4% specificity (accuracy 72.3%) in predicting mortality.[16] Another study found that an MPV cut-off point of 8.5 fL had sensitivity 48.7% and specificity 75.8%.[17] In our study, MPV also showed good accuracy in diagnosing neonatal sepsis with AUC 0.894 (95%CI 0.812 to 0.976). The MPV cut-off point of 7.44 fL was used based on ROC curve analysis. There were 29 subjects (35%) who had MPV ≥7.44 fL.

The MPV cut-off point of 7.44 fL had 80% sensitivity, suggesting that MPV may be a marker of neonatal sepsis. The 84.2% specificity of MPV in our study was also good, and indicated that this MPV cut-off was able to determine that 84.2% of subjects did not have neonatal sepsis. The PPV in our study was 69%, indicating that a positive diagnostic outcome (MPV value ≥7.44 fL) was actually neonatal sepsis 69% of the time. Further examination (such as blood culture test) would be needed to confirm the diagnosis. The NPV in our study was 90.6%, indicating that 90.6% of subjects did not suffer from neonatal sepsis if their MPV value was <7.44 fL, however, we must consider the prevalence of neonatal sepsis in the region because PPV and NPV were influence by prevalence of the disease.

A limitation of this study was its retrospective design. The other weakness was that the media used to grow blood culture microorganisms was only useful for bacteria and fungi. Other causes of neonatal sepsis such as viruses could not be detected. Future studies using a prospective design and blood culture media that can grow bacteria, fungi, or viruses need to be done to further assess the MPV diagnostic value for neonatal sepsis. Our results of the diagnostic test indicate that MPV examination can be used to diagnose neonatal sepsis with 80% sensitivity and 84.2% specificity. The cut-off value of MPV <7.44 fL may be used to exclude a diagnosis of neonatal sepsis (NPV 90.6%).

Conflict of interest

None declared.

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Original Article

Comparison of nutritional status among children with biliary atresia according to age at the time of Kasai procedure

Febricilla Citra Pratiwi, Titis Widowati, Endy P. Prawirohartono

Abstract

Background Recent studies revealed better outcomes among children with biliary atresia (BA) who underwent Kasai procedure at 60 to 90 days of age. Delayed Kasai procedure has a high risk of complications, including nutritional deficits which lead to malnutrition.

Objective To determine the nutritional status of children with BA according to age at the time of Kasai procedure.

Methods Using medical records, we conducted a retrospective study in children with BA based on magnetic resonance cholangiopancreatography (MRCP) or intraoperative cholangiography, who were admitted between 2015 to 2017 and underwent Kasai procedure at Dr. Sardjito General Hospital, Yogyakarta. Nutritional status was evaluated on the day before Kasai procedure, and classified into 4 groups of subjects based on age at the time the children underwent Kasai procedure (<60 days, 60-90 days, >90-120 days, and >120 days). Normal distribution data was analyzed with Saphiro-Wilk test and mean T-test was used to compare mean age at the time of Kasai procedure between groups of well-nourished and malnourished subjects.

Results A total of 39 children with BA underwent Kasai procedure. Of 3 children who underwent Kasai procedure at <60 days of age, 2 were well-nourished and 1 was malnourished. Of the 12 children who underwent Kasai procedure at 60-90 days of age, 6 were well-nourished and 6 were malnourished. Of the 7 children who underwent Kasai procedure at >90-120 days of age, 4 were well-nourished and 3 were malnourished. Of 17 children who underwent Kasai procedure at >120 days of age, 5 were well-nourished and 12 were malnourished. The means of age at the time of Kasai procedure were higher in malnourished subject than well-nourished.

Conclusion The highest prevalence of malnourishment is seen in children with biliary atresia who underwent Kasai procedure at >120 days of age. **[Paediatr Indones. 2019;59:294-7; doi: http://dx.doi.org/10.14238/pi59.6.2019.294-7]**.

Keywords: *biliary atresia; children; nutritional status; Kasai procedure*

Biliary atresia is an obstructive cholangiopathic disease of unknown etiology, characterized by inflammatory destruction of the intra- and extrahepatic bile ducts, which causes bile flow obstruction and leads to cholestasis and cirrhosis.[1,2] The current management of biliary atresia patients is to relieve the extrahepatic biliary obstruction and restore bile flow by performing Kasai procedure while waiting for a liver transplant.[3]

Age of diagnosis plays a major role on biliary atresia management. Delayed Kasai surgery impacts bile retention leading to many severe complications.[3] Malnutrition is the most common complication in untreated biliary atresia patients. It frequently occurs within the first few months of life caused by nutritional deficits. The earlier the malnutrition occurs, the higher risk of a poor prognosis. Therefore,

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the aim of our study was to evaluate the pre-operative nutritional status of biliary atresia patients according to the age at which they underwent Kasai procedure.

Methods

We conducted a retrospective study among children with biliary atresia based on magnetic resonance cholangiopancreatography (MRCP) or intraoperative cholangiography who were admitted between January 2015 and December 2017 and underwent Kasai procedure at Dr. Sardjito General Hospital, Yogyakarta. Data were collected from patients' medical records using a questionnaire covering demographic data (age and sex) as well as clinical data which included date of admisson, date of diagnosis, date of Kasai procedure, type of biliary atresia, weight, and height on the day before Kasai procedure.

Nutritional status was evaluated on the day before Kasai procedure and subjects were classified into well-nourished or malnourished, using weight-for-height z-scores. Malnourished status was defined as having a weight-for-height z-score of less than -3.0 to less than -2.0 based on the WHO Child Growth Standard 2006.[4] Patients with incomplete medical records were excluded from the study. Subjects were divided into four age groups according to their age at the time of Kasai procedure: <60 days, 60-90 days, >90-120 days, and >120 days. Data were analyzed using *SPSS version 20.0* software (*SPSS Inc.*, Chicago, IL, USA). The data of subjects were tested for normal distribution using the Saphiro-Wilk test before calculation of differences. Univariate analysis was conducted to describe the characteristics and distribution of research data. Mean T-test was used to compare mean age at the time of Kasai procedure between groups of well-nourished and malnourished subjects.

A P-value of <0.05 was consindered statistically significance. Results were presented in the form of descriptive narrative and tables. This study received approval from the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Gadjah Mada University/ Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

Results

A total of 39 children who had been diagnosed with biliary atresia and underwent Kasai procedure at Dr. Sardjito General Hospital, Yogyakarta between January 2015 and December 2017 were included in the study. Characteristics of subjects are shown in **Table 1**. Of our subjects, 44% were well-nourished and 56% were malnourished.

Table 1. Basic characteristics of study subjects

Characteristics	N =39
Gender, n	
Male	22
Female	17
Median age of admission (IQR), day	103 (15 - 323)
Median age of Kasai procedure (IQR), day	118 (46 – 324)
Type of biliary atresia, n	
Type 1	2
Type 2	16
Type 3	18
Unclassified	15
Age at Kasai procedure, n	
<60 days	3
60-90 days	12
>90-120 days	7
>120 days	17

Subjects' nutritional status according to age at the time of Kasai procedure is shown in **Figure 1**. These children underwent Kasai procedure at <60 days of ages, 2 out of 3 were well-nourished. Of the 12 patients who underwent Kasai procedure at 60-90 days of age, 6 were well-nourished and 6 were malnourished. Of the 7 patients who underwent Kasai procedure at >90-120 days of age, 4 were well-nourished and 3 were malnourished. Of 17 patients who underwent Kasai procedure at >120 days of age, 5 were well-nourished and 12 were malnourished. The highest proportion of malnourished subjects was in the >120 days of age group.

The means of age at the time of Kasai procedure were significantly higher in malnourished subject than well-nourished (**Table 2**).

Table 2. Univariate analysis of nutritional status according to age at the time of Kasai procedure

Variables	Mean age at the time of Kasai procedure (SD), days	P value
Nutritional status		0.021
Well-nourished	101 (33)	
Malnourished	137 (59)	

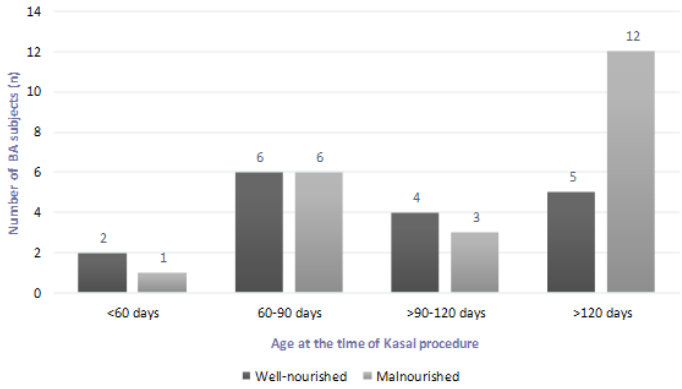

Figure 1. Proportion of nutritional status in BA patients according to age at the time of Kasai procedure

Discussion

The median age of children with biliary atresia who underwent Kasai procedure in our hospital was 118 days, and 92% of subjects underwent Kasai surgery after 60 days of life. Previous studies have confirmed that timing of management of children with biliary atresia, including diagnosis and Kasai procedure, had an important role and was directly correlated to prognosis.[1,3] Children with biliary atresia who undergo Kasai procedure before 60 days of age have a high likelihood of obtaining good initial bile flow (80-90%), improved liver funtion, and fewer complications.[1,2] Increased age at Kasai procedure led to a progressive and lasting detrimental effect on outcome. Obstruction of the biliary tract between the liver and intestines results in bile retention and causes destruction of the liver. Thus, performing the Kasai procedure in older infants reduces its success because of hepatic cirrhosis and greater complications.[3]

Malnutrition is a common complication in biliary atresia patients. Several studies have provided evidence that malnutrition was associated with prognosis in patients with liver disease. Malnourished patients exhibited a significantly increased incidence of post-operative infection, delayed recovery from illness, and increased mortality after surgery.[5] In our study, 56% of biliary atresia patients were malnourished before Kasai procedure, similar to previous studies which reported proportions of 46-53%.[1,5] Biliary atresia patients tend to present with nutritional deficiencies caused by multiple factors, including increased energy and nutrient loss, reduced calorie intake, alteration of nutrient metabolism, and greater nutritional needs. Due to inadequate nutrient intake and increased consumption, biliary atresia patients frequently exhibit protein-energy malnutrition.[5] Children with biliary atresia have poor bile flow, resulting in reduced delivery of bile acids to the small intestine, which impairs micelle formation necessary for absorption of fat-soluble vitamins A, D, E, and K, as well as subsequent fat and fat-soluble vitamin malabsorption.[1,6] In addition, children with biliary atresia have poor appetites and higher resting energy expenditure than healthy normal children.[1]

Malnourishment was found in every age group (**Figure 1**), with greater numbers in the oldest age group. Most of our subjects who underwent Kasai procedure at more than 120 days of age presented with malnutrition, which was evaluated on the day before Kasai procedure. Our results indicate that delaying Kasai procedure to >120 days of age contributes to higher risk of malnutrition complications.

Reasons for delayed Kasai procedure include late recognition and/or referral of biliary atresia patients. Delayed recognition of biliary atresia is caused by the difficulties in differentiating jaundice symptoms from other jaundice disorders and identifying abnormal stool color. A lack of awareness in primary health care workers or physicians for referring children with persistent jaundice for specialty care is also a major problem in most parts of the world, especially in developing countries, including Indonesia. A previous study noted that in India, only 20% cases that presented in most health centers were aged less than 60 days.[7]

A limitation of this study was that we cannot show a causal relationship between variables. With regards to higher prevalence of malnutrition in the older age group, we recommend improving the awareness and ability of our primary healthcare workers or physicians to immediately identify and refer infants with persistent jaundice, as well as to consider the critical importance of nutritional status assessment. Attention should also be paid to metabolic changes in biliary atresia patients. The appropriate nutritional support may reduce the incidence of malnutrition and improve the outcome.

In conclusion, malnourishment in children with BA is higher in those who undergo Kasai surgery at an older age. We note the highest prevalence of malnourishment in biliary atresia patients who underwent Kasai procedure at >120 days of age. Kasai procedure is recommended within 60-120 days of life to attain good nutritional status.

Conflict of interest

None declared.

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Original Article

Triiodothyronin (T3) as a parameter of mortality in sepsis patients in the PICU

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Abstract

Background Thyroid hormone stimulates the regulation of β-adrenergic receptors in order to increase the inotropic effect of the heart myocardium. Euthyroid sick syndrome is a disorder of non-metabolic thyroid function, which is characterized by a decrease in triiodothyronine (T3) levels in patients with non-thyroid systemic disease, such as sepsis. Low serum T3 hormone level is a potentially high-risk factor for mortality from sepsis.

Objective To assess for a relationship between decreased serum T3 levels and mortality in pediatric sepsis patients admitted in the PICU.

Methods This study used a nested case-control design. The subjects were children aged 1 month-18 years who were diagnosed with sepsis in the pediatric intensive care unit at Sanglah Hospital, Denpasar, Bali, from September 2017 to January 2019.

Results A total of 90 children were included, of whom 44 died and 46 survived. Median age was 10.5 (IQR 44) months in subjects who died and 9 (IQR 50) months in those who survived. The majority of subjects in both groups had well-nourished nutritional status. Bivariate analysis revealed that significantly more subjects who died had low serum T3 (≤1 ng/dL), PELOD-2 score ≥5, than subjects who survived. Multivariate analysis revealed that serum T3 ≤ 1 ng/dL (OR 55.1; 95%CI 9 to 334.8; P<0.001) and PELOD-2 score ≥ 5 (OR 6.5; 95%CI 1.6 to 26.7; P=0.01) were significant risk factors for sepsis mortality.

Conclusion Low serum T3 level and high PELOD-2 score are risk factors for death in sepsis. **[Paediatr Indones. 2019;59:298-302; doi: http://dx.doi.org/10.14238/pi59.6.2019.298-302]**.

Keywords: *sepsis; euthyroid sick syndrome; outcome; T3 serum*

Sepsis is a major cause of infant and child mortality. It is a systemic disease caused by the spread of microbes or toxins into the bloodstream leading to a systemic response. The cause of death in septic patients depends on various factors, such as the type of bacteria, duration from the occurrence of illness until treatment onset, primary illness before the sepsis occurred, and the current immunization status of the patient.[1] Leon-Sanz *et al*.[2] performed a cross-sectional study about the relationships between thyroid status and prevalence, therapy, outcome, risk of failed therapy, and length of stay in septic patients. They showed a difference in septic characteristics between pediatric and adult patients. Malnutrition was also one of the factors that can affect the risk of death in sepsis. Other factors that can affect the outcome of sepsis were age, poor nutritional status, prematurity, invasive treatment, chronic diseases, and immunodeficiency.[2]

Serum T3 level influences the compensating body response to chronic disease and sepsis. Serum

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T3 activates stress hormones as well as increases the effectiveness of β-adrenergic receptors, as an inotropic activator of cardiac smooth muscle.[3] In recent years, more study has been done on the relationship between non-metabolic thyroid dysfunction (euthyroid sick syndrome) and mortality in septic patients. Decreased T3 levels in blood were found to increase the risk of death three times higher in septic patients.[3]

A previous study reported that T3 had an important role in determining mortality rate in European geriatric patients with euthyroid sick syndrome who had sepsis and other chronic diseases.[4] Angeloussi *et al.*[5] in Europe conducted a systematic review of nine prospective cohort studies, comprised of studies on adults (2), neonates (3), and children (4). They found a significant correlation between septic patient outcome and reduced T3 levels in blood.[6] However, a prospective cohort study in India found no significant relationship between hormone levels and survival rate in children with sepsis.[5]

Demographic and climate differences between Indonesia and Europe can affect T3 levels. In Indonesia, efforts to meet iodine intake requirements have not been uniform throughout the country, and may be a cause of T3 deficiency in blood. A study in Semarang, Indonesia reported no significant difference between thyroid hormone levels and outcome in septic patients. But they used a cross-sectional method with a small sample size, so the results cannot be generalized to the entire population.[7]

Since Indonesia has different demographicss, climates, and populations compared to Europe, European study results may not be applicable to Indonesia. In addition, considering the dearth of research conducted specifically in children, and the relatively small sample size in Indonesian studies, we aimed to further assess if T3 levels in blood are a risk factor of mortality in children with sepsis.

Methods

This study used a nested case-control study design. We calculated the sample size using unpaired, categorical, comparative analytic sample, taking into account a possible 10% drop out rate. Subjects were included by consecutive sampling. Ninety subjects' blood specimens were taken by laboratory personnel trained at the Sanglah Laboratory Denpasar, Bali, at first day of sepsis diagnosis, and stored at -200C in the laboratory. Age was divided into 3 groups based on epidemiology : <12 month, 12-144 month, >144 months. Nutritional status was the ratio between current body weight in kg divided by ideal weight plotted in the CDC and WHO curve, and classified into obesitiy, well-nourished, moderate malnutrition, severe malnutrition, and failure to thrive.

Procalcitonin was analysed using the ELISA method (ng/mL) and categorized as > 36 ng/mL and ≤ 36 ng/mL. Procalcitonin data was taken from medical record. The PELOD-2 was a scoring system used to determine the risk of mortality patients based on the laboratory data on medical record, classified as score ≥5 or <5. All data was taken at the time of initial diagnosis and subjects' conditions were followed until the end of treatment, then they were divided into the died (case) and survived (control) groups. Serum T3 levels in both groups were measured by electrochemiluminescent immunoassay (ECLIA) method (ng/dL), and categorized as low (≤ 1 ng/dL) or normal (> 1 ng/dL).

All data were analyzed with *SPSS v.21*. We conducted bivariate analysis using the Chi square test. If P value was <0.002 we continued to multivariate analysis using logistic regression. This study was approved by the Ethics Committee of Universitas Udayana Medical School/Sanglah Hospital, Denpasar.

Results

This was a nested case-control study of 90 children aged 1 months-18 years who were treated in the PICU Sanglah Hospital Denpasar, Bali, from September 2017 until the samples was fulfilled in January 2019. The flow of study results can be seen from **Figure 1**.

The charateristics of the subjects based on the group of not survive and survive showed procalcitonin level in not survive (died) at median of 63 (IQR 88.9) and 22(IQR 35.8) in survive. The T3 serum in died was 0.7 (IQR 0.38) and 1.4(IQR 0.83) in survived groups. The PELOD-2 score was 4(IQR 4) in died groups, and 2(IQR 2) in survived. The characteristics of full subjects are shown in **Table 1**.

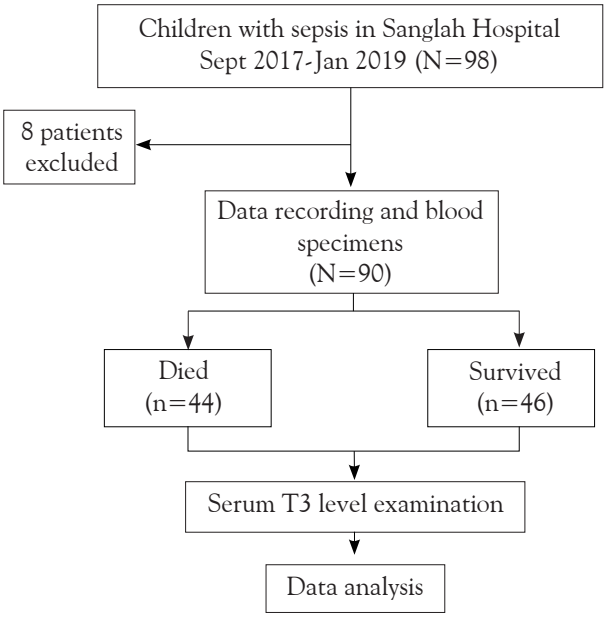

Figure 1. Flow chart of the study scheme

Table 1. Characteristics of subjects by outcomes

Characteristics	Died (n=44)	Survived (n=46)
Median age (IQR), months	10.5 (44)	9 (50)
Age, n (%)		
<12 mo	23 (52.3)	27 (58.7)
12-144 mo	20 (45.5)	16 (34.8)
>144 mo	1 (2.3)	3 (6.5)
Sex, n (%)		
Male	22 (50)	22 (50)
Female	25 (54.3)	21 (45.7)
Nutritional status, n (%)Obese		
Well-nourished	2 (4.5)	2 (4.3)
Moderate malnutrition	20 (45.5)	23 (50)
Severe malnutritioN	13 (29.5)	17 (37)
Failure to thrive	5 (11.4)	1 (2.2)
	4 (9.1)	3 (6.5)
Median procalcitonin (IQR), ng/mL	63 (88.9)	22 (35.9)
Median T3 serum (IQR), ng/dL	0.7 (0.38)	1.4 (0.83)
Median PELOD-2 score (IQR)	5 (4)	2 (2)

Bivariate analysis were analysed using Chi-square test, with normality test using Kolmogorov Smirnov and Shapiro-Wilk tes for data distribution. From bivariate analysis there was two component which had P value < 0,002 which were serum T3 and PELOD-2. The all results of bivariate analysis are shown in **Table 2**.

Table 2. Bivariate analysis of sepsis outcomes and variables

Variables	Groups		OR	95%CI	P value
	Died (n=44)	Survived (n=46)			
Serum T3, n (%)					
Low	42 (95.5)	13 (28.3)	53.3	11.236 to 252.910	<0.001
Normal	2 (4.5)	33 (71.7)			
PELOD-2 score, n (%)					
≥5	26 (59.1)	5 (10.9)	11.8	5.9 to 35.8	<0.001
< 5	18 (40.9)	41 (89.1)			
Procalcitonin, n (%)					
>36 ng/mL	28 (63.6)	14 (30.4)	4	1.66 to 9.63	0.002
≤36 ng/mL	16 (36.4)	32 (69.6)			
Sex, n (%)					
Male	22 (50)	25 (54.3)	0.8	0.3 to 1.9	0.8
Female	22 (50)	21 (45.7)			
Age, n (%)					
<12 months	23 (52.3)	27 (58.7)	1	0.4 to 2.4	0.9
12-144 months	20 (45.4)	16 (34.8)	0.9	0.6 to 1.5	0.5
> 144 months	1 (2.3)	3 (6.5)	Ref	-	-
Nutritional status, n (%)					
Obese	2 (4.5)	2 (4.3)	0.8	0.5 to 1.3	0.6
Well-nourished	20 (45.5)	23 (50.0)	Ref	-	-
Moderate malnutrition	13.(29.5)	17 (37.0)	0.7	0.2 to 1.7	0.4
Severe malnutrition	5 (11.4)	1 (2.2)	0.7	0.7 to 1.7	0.8
Failure to thrive	4 (9.1)	7 (7.8)	0.8	0.8 to 1.4	0.6

From multivariate analysis we found low T3 serum level and high of PELOD-2 score were high predictors to dead in sepsis patient (P<0.001) (**Table 3**).

Table 3. Multivariate analysis

Variables	Step I			Step IV		
	Adjusted OR	95% CI	P value	Adjusted OR	95% CI	P value
Low serum T3 (≤1 ng/dL)	56.4	8.7 to 364.4	<0.001	55.1	9 to 334.8	<0.001
PELOD-2 score (≥5)	5.5	1.3 to 23.5	0.02	6.5	1.6 to 26.7	0.01

Discussion

Median serum T3 level in septic patients who died was 0.7 (IQR 0.38)ng/dL. Bivariate analysis revealed that 95.5% of these subjects had serum T3 ≤ 1 ng/dL, while only 28.3% did in the survived group. Further multivariate analysis revealed that serum T3 ≤ 1 ng/dL had a significant association with mortality (OR 55.1; 95% CI 9-334.8; P <0.001). Our results were in agreement with a systematic review performed in Europe who analyzed 7 pediatric studies and 2 adult studies. They showed that decreased total T3 level below 1 ng/dL increased the risk of death in 8 studies with OR 38-42, while 1 study did not have a significant correlation.[5] Previous studies also noted no significant relationship between thyroid hormone levels in septic patients and outcomes. This result could have been affected by their smaller sample sizes of 30 and 49 subjects, respectively.[6,7]

The Pediatric Logistic Organ Dysfunction (PELOD)-2 is an updated scoring system for assessing organ dysfunction in critically ill patients in order to predict mortality in septic patients. We found that sepsis patients who died had the median PELOD-2 score of 5 (IQR 4). Bivariate analysis revealed that significantly more sepsis patients with PELOD-2 score >5 died than survived (59.1% *vs.* 10.9%, respectively; (P<0.001). Multivariate analysis revealed that septic patients with PELOD-2 score ≥ 5 had a significantly higher risk of mortality than those with score of <5 (OR 6.5; 95%CI 1.6 to 26.7; P=0.01). In contrast, a previous study found that the PELOD-2 score had to be more than 20 to indicate higher risk of mortality in sepsis patients. This difference may have been due to their larger sample size of 209 subjects.[8]

The PELOD-2 score analysis was also in line with a study conducted at Sanglah Hospital, Denpasar. Daily PELOD scores were used as predictors of mortality. They found that higher PELOD score was associated with the faster mortality. They also noted that low PELOD score occured on day 23, moderate score on day 12, and high score on day 7.[9] The difference in our study was that we only evaluated PELOD-2 score on the first day of the treatment, not daily, and .the previous study used PELOD as predictors, but from our study we used PELOD-2 score which modified from PELOD score.

A limitation of our study was that serum T3 level was measured only at the beginning of treatment, not when the patient experienced the outcome. Also, the primary diseases in this study were not considered as one of the factors to cause death in sepsis.

Our results provide evidence that septic patients with decreased serum T3 levels and high PELOD-2 score are at greater risk of mortality. As such, low T3 level can be used as a risk factor of mortality in septic patients, but must be supported by the evidence of organ failure.

In conclusion, low serum T3 (< 1 ng/dL) and high PELOD-2 score (>5) were significant risk factors for mortality in pediatric sepsis patients. Procalcitonin is not a significant risk factor for mortality.

Conflict of interest

None declared.

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Original Article

Current management of children with acute otitis media: a feasibility survey for a pragmatic study

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Abstract

Background Acute otitis media (AOM) is a common self-limiting infection where antibiotics confer limited benefit. Other treatments, such as anti-inflammatory agents have been proposed as an alternative to antibiotics, but no high-quality clinical trials have tested this.

Objective To identify current AOM management practices among Indonesian clinicians. We also required this information for our proposed corticosteroids clinical trial for AOM.

Methods This cross-sectional study surveyed a convenience sample of general practitioners (GPs), pediatricians, and Ear-Nose-Throat (ENT) specialists in Jakarta, Depok, and Bekasi. We addressed their current AOM management practices and willingness to participate in a future trial on corticosteroids.

Results We distributed 2,694 questionnaires through conferences, primary care/hospital visits, and by mail-list group. Of 492 questionnaires received (response rate 18%), 352 were from eligible clinicians. Most clinicians diagnosed AOM by using an otoscope (64-91%). Tympanometry was used by a quarter of ENT specialists. Amoxicillin-clavulanate was the most common antibiotic for AOM, prescribed by pediatricians and ENT specialists, whilst most GPs prescribed amoxicillin. Clinical scenarios indicated most ENT specialists (88%) would prescribe antibiotics and most pediatricians (54%) would choose expectant observation by withholding antibiotics for mild AOM. Almost half of clinicians would consider using corticosteroids in a trial.

Conclusion Most clinicians would prescribe antibiotics for mild AOM. However, slightly over half of pediatricians would solely choose expectant observation. Adequate numbers of potential participating clinicians, who would consider using corticosteroids, make our proposed corticosteroids trial for AOM feasible. We found gaps between clinical practice and evidence requiring further investigation to improve AOM management in Indonesia. **[Paediatr Indones. 2019;59:303-17; doi: http://dx.doi.org/10.14238/pi59.6.2019.303-17]**.

Keywords: *otitis media; acute disease; anti-bacterial agents; health services; survey and questionnaires*

Antibiotics are commonly used for treating infectious diseases, including acute otitis media (AOM) in children.[1,2] International guidelines recommend expectant observation for mild AOM, with antibiotics only given for severe cases.[3,4] Although antibiotics are effective for AOM, their effects in improving pain and middle ear effusion are weak, they have potentially harmful side effects, and may lead to antibiotic resistance.[5,6] Currently, there are two conflicting Indonesian guidelines for AOM. One recommends antibiotics for both mild and severe AOM, while the other recommends antibiotics only for severe cases (although 'severe' is not clearly defined).[7,8] This potentially contributes to high use of antibiotics in the management of AOM in Indonesia, thereby

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increasing the risk of antibiotic resistance and other side effects.[5,9,10] Finding alternative treatment options could be one way to mitigate the risk of antibiotic resistance. Theoretical considerations suggest that the anti-inflammatory effect of corticosteroids might reduce symptoms.[1] Conflicting evidence on the potential benefits requires a large randomized placebo-controlled trial (RCT) to test efficacy.[12,13] We plan to undertake a large RCT in Indonesia to further investigate this issue.

To date, there is no available data on the current AOM management for children in Indonesia. However, there have been several studies surveying the management of AOM in other countries.[14-16] A survey study in Turkey showed pediatricians were likely to prescribe antibiotics (60%) and analgesics (e.g., acetaminophen, ibuprofen) for children with AOM.[14] Surveys in India and Israel showed most ENT specialists prescribed antibiotics for AOM (62-98%). Analgesics and decongestants and/or antihistamines were also commonly prescribed. Most ENT specialists in India used an otoscope as a diagnostic tool for AOM, whilst a microscope was more preferable in Israel.[15,16]

We identified the current management, particularly with regards to prescribing antibiotics for AOM in three cities in Indonesia. This survey study was done to help us identify existing gaps between clinical practice and evidence in the management of AOM in Indonesia. Our survey was also designed to gauge clinicians' willingness to use corticosteroids in a future, randomized trial.

Methods

This study primarily aimed to identify the current management of AOM among clinicians. It also identified the feasibility of a proposed clinical trial to test corticosteroids for AOM in children. We conducted a cross-sectional study for our survey (April - August 2016). Our eligibility criteria were clinicians from three specialties (general practitioners, pediatricians, and ENT specialists) working in primary/secondary or tertiary healthcare facilities in Jakarta, Depok, and Bekasi, Indonesia. We established clinicians' specialties and email addresses from the national professional organizations of general practitioners (*Indonesian Medical Association*), pediatricians (*Indonesian Pediatric Society*), and ENT specialists (*Indonesian Otorhinolaryngology Head and Neck Surgery Society*). We then distributed paper-based questionnaires through workshops, conferences, primary healthcare and hospital visits. We also distributed electronic-based questionnaires through mailing lists of primary care clinician graduates from two medical schools in Jakarta (Universitas Indonesia and Universitas Pembangunan Nasional Veteran), identified by alumni and colleagues.

We invited participation in a 10-minute presentation at the following workshops and conferences: (i) The Indonesian National Committee for the Prevention and Management of Hearing Impairment and Deafness Meeting (20 May 2016), (ii) The Continuing Professional Development Program: The Comprehensive Management of Vestibulocochlear Disorders (20-21 May 2016), (iii) The Third Neurotology Update Management: Hearing and Vestibular Disorders in Children (21 May 2016), (iv) The Annual Scientific Meeting of the Indonesian Medical Association (27-29 May 2016), and (vi) The Second Jakarta Pediatric Respiratory Forum (29-30 May 2016). We distributed the questionnaires at the registration table on the first day of the workshops or conferences and collected the questionnaires at the end of the events.

We identified several primary healthcare clinics and hospitals located in Jakarta, Depok, and Bekasi that were conveniently accessible. We contacted the heads or directors of the appointed healthcare facilities and distributed the questionnaires to the emergency, pediatric, and otorhinolaryngology departments. We collected the questionnaires at most 4 weeks after their distribution, unless completed earlier.

Consenting clinicians typically completed the questionnaires in 10-20 minutes (**Appendix 1**). It had 3 sections: 1) current management of AOM in children, including diagnostic treatment items; 2) 3 clinical scenarios [(a.) a child aged < 2 years with mild AOM; (b.) a child aged ≥ 2 years with mild AOM; and (c.) an older child with recurrent and bilateral AOM] in which respondents were invited to describe their management (**Table 1**); and 3) a feasibility survey to identify the number of pediatric AOM patients and clinicians who might be willing to participate in our clinical trial on corticosteroids as an alternative treatment for AOM.

We did not formally determine a sample size estimate. We used convenience sampling based on ease of accessibility to the healthcare facilities and clinicians who attended workshops and conferences specifically for general practitioners, pediatricians, and ENT specialists. The results of this study are reported as the percentage of clinicians in each category of responses. We used Chi-square test to identify the differences between specialty groups using *IBM SPSS Statistics 23* software. All proportions are expressed from respondents who answered that question in the questionnaires.

This study protocol was reviewed and approved by the Ethics Committee of the Universitas Indonesia Medical School and Bond University Human Research Ethics Committee. We provided a paper- and electronic-based information sheet and consent form to all respondents. Consent was received in written form and the consent process was free of coercion. All information obtained from the respondents was treated as confidential.

Results

Of 2,694 questionnaires distributed, 492 were returned (response rate 18%). Of these, 445 (90%) participated in the survey, **Figure 1**. There were 352

Table 1. Clinical scenarios and their interpretation

Clinical scenarios	Interpretation/diagnosis	Treatment recommendation[3]
Clinical scenario 1: "A one-year old boy, accompanied by his mother, came to your practice with a complaint of pain in his left ear for one day. The pain was not severe. He has had a cold for the last two days with a mild fever. At the physical examination, he looked well and alert with temperature 37.8°C. At his ear, nose, and throat examination, there was mucous discharge on the nasal cavities, and his throat looked normal. An otoscopic examination showed redness and bulging tympanic membrane of the left ear."	A young child (aged < 2 years) with mild AOM	Expectant observation (begin antibiotics if child worsens or fails to improve within 48 to 72 hours of AOM onset) OR antibiotic therapy if close observation and follow-up cannot be ensured.
Clinical scenario 2: "A four-year old girl, accompanied by her parents, came to your practice with a complaint of pain in her right ear for one day. She has had a cold for the last four days. She had no fever. At the physical examination, the patient looked well and alert. At her ear, nose, and throat examination, there was serous secretion in the nasal cavities and her throat looked normal. An otoscopic examination showed redness and bulging tympanic membrane of the right ear."	An older child (aged ≥ 2 years) with mild AOM	Expectant observation (begin antibiotics if child worsens or fails to improve within 48 to 72 hours of AOM onset) OR antibiotic therapy if close observation and follow-up cannot be ensured.
Clinical scenario 3: "A five-year old girl, accompanied by her parents, came to your practice with a complaint of pain in her right ear for one day, followed by left ear this morning and she had a mild fever. She had experienced acute otitis media in her right ear one month ago. At the physical examination, the patient looked well and alert with temperature 36.8°C. At her ear, nose, and throat examination, there was minimal serous discharge in her nasal cavities and her throat looked normal. An otoscopic examination showed redness and bulging on both tympanic membranes."	An older child with recurrent bilateral AOM	Expectant observation (begin antibiotics if child worsens or fails to improve within 48 to 72 hours of AOM onset) OR antibiotic therapy if close observation and follow-up cannot be ensured. However, this guideline does not specify the need of antibiotics for recurrent AOM.

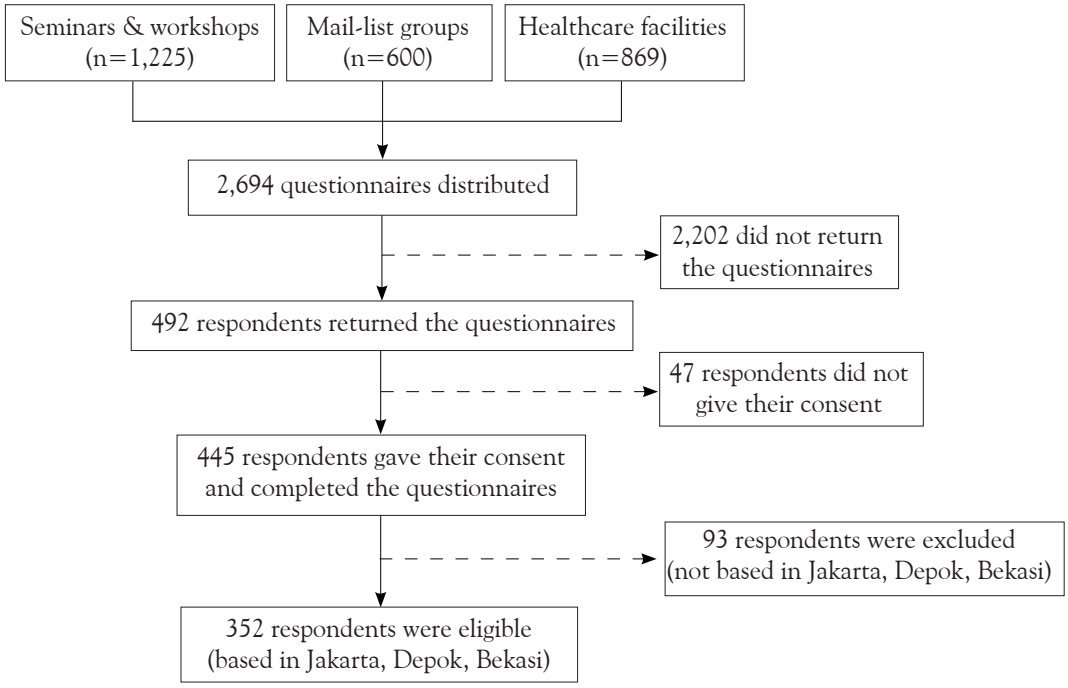

Figure 1. A flow diagram of study recruitment

clinicians who responded (general practitioners 81%, pediatricians 10%, and ENT specialists 9%) and they were based at primary/secondary (44%) and tertiary healthcare (54%) facilities (no response 2%) in Jakarta (82%), Depok (10%), and Bekasi (8%), our target regions. Most were young, <31 years (44%), and female (67%).

A total of 705 children with AOM visited 352 clinicians over a period of seven days prior to answering the questionnaire. Most clinicians had less than three AOM cases during this period (75%), and most of these children were between two and five years of age. ENT specialists had more AOM cases (three to five cases) per week compared to general practitioners and pediatricians.

Most clinicians diagnosed AOM by using an otoscope (64-91%) (**Table 2**). Three quarters of ENT specialists and 9% of general practitioners used an endoscope. Pediatricians did not use an endoscope. A quarter of ENT specialists, but very few pediatricians (3%) or general practitioners (1%), used tympanometry to identify middle ear effusion. Few clinicians (0-6%) used a pneumatic otoscope, audiometry (0-9%), or tympanocentesis (0-3%) to diagnose AOM.

Amoxicillin-clavulanate was the most common antibiotic prescribed by ENT specialists (58%), pediatricians (46%), and general practitioners (16%) (**Table 2**). Amoxicillin was the second most common antibiotic prescribed by general practitioners (43%) and pediatricians (37%), whilst ENT specialists prescribed cefixime as the second common antibiotics for AOM (23%). Azithromycin (0-13%), cefadroxil (0-9%), erythromycin (3%), ampicillin (0-3%), and cotrimoxazole (0-3%) were the least common antibiotics prescribed for AOM. General practitioners (66%) mostly prescribed amoxicillin for three to five days, whilst ENT specialists (56%) and pediatricians (54%) prescribed amoxicillin-clavulanate for more than five days.

The clinicians' treatment choices for the three clinical scenarios are shown in **Table 3**. In the first scenario, 88% of ENT specialists would prescribe antibiotics for AOM, followed by general practitioners (71%) and pediatricians (57%). More ENT specialists (44%) would prescribe corticosteroids when compared to other specialties (general practitioners 30%; pediatricians 23%). In the second scenario, 66% of ENT specialists would prescribe antibiotics, followed by general practitioners (52%) and pediatricians

Table 2. The common diagnostic tools and type of antibiotic prescribed among clinical specialties

Management	General practitioners* (Total=284), n(%)	ENT specialists (Total=32), n (%)	Pediatricians (Total=35), n(%)
Diagnostic examination			
Otoscope	183 (64)	29 (91)	24 (69)
Penlight/headlamp	159 (56)	9 (28)	20 (57)
Endoscope	25 (9)	24 (75)	0 (0)
Tympanometry	2 (1)	8 (25)	1 (3)
Tuning fork	10 (4)	4 (13)	0 (0)
Pneumatic otoscope	8 (3)	0 (0)	2 (6)
Tympanocentesis	1 (1)	3 (9)	0 (0)
Audiometry	4 (1)	1 (3)	0 (0)
Type of antibiotics**			
Amoxicillin-clavulanate	46 (16)	18 (58)	16 (46)
Amoxicillin	122 (43)	0 (0)	13 (37)
Cefixime	34 (12)	7 (23)	2 (6)
Azithromycin	4 (1)	4 (13)	0 (0)
Cefadroxil	26 (9)	0 (0)	0 (0)
Erythromycin	9 (3)	1 (3)	1 (3)
Ampicillin	4 (1)	0 (0)	1 (3)
Cotrimoxazole	8 (3)	0 (0)	0 (0)
Others	6 (2)	0 (0)	0 (0)
More than one	22 (8)†	1 (3)‡	2 (6)§

*One missing data in general practitioner group

**Total 281 general practitioners and 31 ENT specialists provided their options of antibiotic types for AOM

†General practitioners chose amoxicillin (77%, 17/22), followed by cefixime (32%, 6/22) and cefadroxil (32%, 6/22) in the multiple antibiotic prescription group.

‡ENT specialist did not mention any antibiotic by name but only reported 'depends on the condition'.

§Pediatricians chose cefixime (100%, 2/2), followed by amoxicillin (50%, 1/2) and amoxicillin-clavulanate (50%, 1/2) in the multiple antibiotic prescription group.

Table 3. Treatment options for three clinical scenarios among all specialties

Treatment options	Scenario 1 A young child (aged < 2 years) with mild AOM, n(%)				Scenario 2 An older child (aged ≥ 2 years) with mild AOM, n(%)				Scenario 3 An older child with recurrent bilateral AOM, n(%)			
	GP (n=283)	ENT (n=32)	PED (n=35)	Overall (n=350)	GP (n=280)	ENT (n=32)	PED (n=35)	Overall (n=347)	GP (n=279)	ENT (n=32)	PED (n=35)	Overall (n=346)
Expectant observation	140 (49)	8 (25)	20 (57)	168 (48)	136 (49)	9 (28)	17 (49)	162 (47)	132 (47)	8 (25)	16 (46)	156 (45)
Antibiotics	200 (71)	28 (88)	20 (57)	248 (71)	147 (52)	21 (66)	16 (46)	184 (53)	170 (61)	27 (84)	20 (57)	217 (63)
Corticosteroids	85 (30)	14 (44)	8 (23)	107 (31)	73 (26)	12 (37)	7 (20)	92 (27)	95 (34)	14 (44)	11 (31)	120 (35)
Acetaminophen	217 (77)	19 (59)	31 (89)	267 (76)	151 (54)	12 (37)	22 (63)	185 (53)	128 (46)	13 (41)	18 (51)	159 (46)
Ibuprofen	49 (17)	9 (28)	8 (23)	66 (19)	74 (26)	11 (34)	9 (26)	94 (27)	78 (28)	9 (28)	13 (37)	100 (29)
Decongestant/ antihistamine	201 (71)	29 (91)	24 (69)	254 (73)	222 (79)	30 (94)	29 (83)	281 (81)	145 (52)	29 (91)	21 (60)	195 (56)
Topical antibiotics	71 (25)	2 (6)	7 (20)	80 (23)	69 (25)	2 (6)	6 (17)	77 (22)	73 (26)	2 (6)	11 (31)	86 (25)
Topical analgesics	32 (11)	0 (0)	4 (11)	36 (10)	33 (12)	1 (3)	3 (9)	37 (11)	41 (15)	1 (3)	5 (14)	47 (14)
Physiotherapy	18 (6)	2 (6)	2 (6)	22 (6)	19 (7)	1 (3)	4 (11)	24 (7)	17 (6)	1 (3)	1 (3)	19 (6)

*Clinicians may choose more than one treatment option

(46%). Thirty-seven per cent of ENT specialists and only up to quarter of general practitioners (26%) and pediatricians (20%) would choose corticosteroids. In the third scenario, 84% of ENT specialists would prescribe antibiotics for AOM, followed by general practitioners (61%) and pediatricians (57%). Corticosteroids were more likely to be prescribed by ENT specialists (44%) compared to other specialties (general practitioners 34%, pediatricians 31%).

Clinicians would mostly prescribe acetaminophen (59-89%), decongestant/antihistamine (69-91%), and antibiotics (57-88%) for a young child with

mild AOM (Scenario 1) compared with other treatment (e.g., ibuprofen, corticosteroids, topical antibiotics, or topical analgesics). From all clinical scenarios, ENT specialists were more likely to prescribe corticosteroids (37-44%) and were less likely to choose expectant observation (25-28%), whereas pediatricians (46-57%) followed by general practitioners (47-49%) were more likely to choose expectant observation compared to ENT specialists. However, as clinicians may choose more than one treatment for the clinical scenario section, there were respondents who chose both expectant observation and antibiotics, which by definition should be mutually exclusive. By identifying those who solely chose expectant observation by withholding antibiotic treatment, pediatricians (43-54%) were still more likely to choose expectant observation compared to other specialties in all three scenarios. The rate of corticosteroid prescribed by pediatricians and general practitioners ranged from 20% to 34% across the three scenarios. A significant difference between specialty groups was identified in the first scenario, where pediatricians were significantly less likely to prescribe antibiotics (P=0.024) compared to general practitioners and ENT specialists, and in the third scenario where ENT specialists were more likely to prescribe antibiotics (P=0.026) compared to other specialties. With regards to prescribing antibiotics in each scenario among these specialties, ENT specialists were more likely to prescribe antibiotics compared to other specialties, particularly in the first and third scenarios.

As shown in **Figure 1**, of the 352 clinicians from Jakarta, Depok, and Bekasi, 171 respondents (49%) indicated their willingness to participate in our proposed clinical trial testing corticosteroids as an alternative treatment for AOM in children. Their characteristics were similar to the whole survey sample. They managed 443 children with AOM in a week. Most clinicians had less than three cases of AOM during this period (75%), with most of these children aged older than five years. ENT specialists had more AOM cases (three to five cases) per week compared to other specialties. These clinicians had a similar practice in the management of AOM and responses to clinical scenarios to the whole sample. Up to 44% of clinicians, mostly ENT specialists, would consider using corticosteroids in a trial.

Discussion

Our sample of clinicians who worked in Jakarta, Depok, and Bekasi, reported that they mostly diagnosed AOM by using an otoscope. Most general practitioners would prescribe amoxicillin for a short duration (3-5 days), whilst ENT specialists and pediatricians were more likely to prescribe amoxicillin-clavulanate for a longer duration. Clinical scenario results showed there was a high rate of antibiotics prescribed for mild AOM. Up to 44% of clinicians would have prescribed corticosteroids for children with AOM. Both corticosteroids and antibiotics were mostly prescribed by ENT specialists in the scenarios. There is no clear justification for a high rate of antibiotic prescribing by ENT specialists. As ENT specialists saw more patients with AOM in the sampled week compared to other specialties, their contribution to antibiotic prescribing would be higher and, therefore, the risks correlated with antibiotic use, such as adverse events and antibiotic resistance would be increased.[5,9,10] Another reason for this high use was due to potential complications following AOM, such as spontaneous perforation of the tympanic membrane (15%) and persistent middle ear effusion (25%).[17,18] High rates of antibiotic prescribing by general practitioners might be influenced by the Indonesian practice guideline recommending antibiotics for both mild and severe AOM.[7] Interestingly, we found pediatricians were less likely to prescribe antibiotics for all scenarios in the study. One potential justification for this was that international pediatrics practice guidelines do not recommend antibiotics for common colds and only recommend antibiotics for AOM with high risks (e.g., children with severe signs and symptoms, children < 2 years with bilateral AOM, or tympanic membrane perforation).[3,4,19] Corticosteroids and antibiotics were more likely to be prescribed for AOM in younger children and recurrent bilateral AOM. These are not entirely in accordance with the guidelines, as corticosteroids have not been recommended by any guidelines. The guidelines only recommend the use of antibiotics for children under two years of age with bilateral AOM, whilst our scenario was a case of unilateral AOM.[3]

Unfortunately, few clinicians (<7%, and none of the ENT specialists) used pneumatic otoscopy, which enables the assessment of tympanic membrane

mobility.[3,20] A systematic review showed that a pneumatic otoscope performed by a skilled clinician can accurately diagnose AOM with high predictive values. It can replace tympanometry as one diagnostic tool for AOM, as the pneumatic otoscope is a more affordable.[21] Our clinical scenarios demonstrated that most clinicians would prescribe antibiotics over expectant observation for mild AOM. Evidence recommends expectant observation with sufficient pain management for mild AOM.[3-5,9] Only 30% of AOM cases are severe and require antibiotic treatment.[5,9] However, antibiotic prescribing rates for AOM in developed and developing countries are still relatively high. In terms of data from general practices in Australia over five years, 89% of new AOM cases were managed with antibiotics.[22] Meanwhile, data from the *National Ambulatory Medical Care Survey* (NAMCS) demonstrated 83.1% of children with isolated AOM were managed with antibiotics.[23] Our study demonstrated that up to 88% of clinicians would prescribe antibiotics for a mild case of AOM. *The Indonesian Practice Guideline* recommends antibiotics for both mild and severe AOM, which may influence the high rate of antibiotic prescribing for AOM in Indonesia.[7] A red and bulging tympanic membrane could be the other reason for antibiotic treatment in all scenarios. However, the sign of red tympanic membrane is not sensitive (18%), with a low likelihood ratio for a positive result of 1.1, regardless of its high specificity (84%). Although a bulging tympanic membrane will help make the diagnosis, it requires the combination of cloudiness and the impaired mobility of the tympanic membrane to robustly diagnose AOM.[20]

Recurrent AOM was the second most common reason for antibiotic prescribing in this study. This is defined as "the occurrence of 3 or more episodes of AOM in a 6-month period or the occurrence of 4 or more episodes of AOM in a 12-month period that includes at least 1 episode in the preceding 6 months".[3] *The American Academy of Pediatrics Clinical Practice Guideline* does not recommend the use of prophylactic antibiotics for reducing the number of episodes of AOM in recurrent AOM cases, and yet tympanostomy tubes should be offered.[3] Systematic reviews showed that recurrent AOM is not included as one indicator for antibiotic treatment in the management of AOM.[5,9] However, *The 2014 New South Wales Guideline* includes recurrent AOM into the high-risk middle ear infection category, which requires immediate antibiotic treatment. [24]

The high rate of antibiotic prescribing in AOM indicates the need for alternative non-antibiotic treatment for AOM. We propose to test corticosteroids for AOM. Inflammation has been indicated as an important mechanism in AOM, despite the complexity of the pathophysiology of AOM. This involves both cellular and chemical mediators (e.g., cytokines, chemokines, mast cells, and leukotrienes). Corticosteroids could act as an anti-inflammatory agent, particularly in the middle ear,[12] and therefore reduce pain. Insufficient evidence of the effects of corticosteroids for AOM requires a large, high-quality, clinical trial to evaluate corticosteroid efficacy to improve the resolution of AOM, as a monotherapy in mild cases or as an addition to antibiotic therapy in severe cases.[12]

Our feasibility survey demonstrated that there were 171 clinicians who were willing to participate in our clinical trial and who saw 443 children with AOM in a week. They also had similar practices in the management of AOM and responses to clinical scenarios to the whole sample. This study also demonstrated that a sufficient number of clinicians would consider using corticosteroids for AOM. This finding was surprising; however, this high rate could be primed by clinicians' knowledge of the nature of the upcoming clinical trial, which had been provided in the consent form and at the conference presentations prior to the completion of the questionnaires. As nearly half of the clinicians were interested in participating in our trial, it is feasible to conduct a clinical trial on corticosteroids for AOM in children in Indonesia, according to the pre-specified timeline (12 months, including a three-month follow-up).

We believe this the first survey of current practices of clinicians in the management of pediatric AOM in Indonesia, which was the strength of this study. We identified clinicians' preferred treatment options, particularly in choosing corticosteroids, expectant observation, and antibiotics. However, this study also had several limitations, including the low response rate, unclear definition of 'observation for 48 to 72 hours' as one answer option in the clinical scenario, and a narrow study-site coverage. We tried several recruitment strategies to increase the response rate, however, as participation was voluntary, it was

the respondents' decision to consent and participate in this survey study. There is no gold standard for an acceptable minimum survey response rate, however, a response rate of at least 70% is desirable.[25] Nonetheless, surveys involving voluntary clinicians mostly have low response rates (< 30%).[25] There are several factors that influence the willingness of clinicians to participate in a survey study, such as concerns about disruption of their practice, time, and relevance of the survey topic.[26] A review assessing survey response rates of general practitioners from published primary care journals demonstrated that the mean response rate was 61% (95% confidence interval 59% to 63%).[27] A cross-sectional study comparing the response rates between postal and online survey of general practitioners across Australia showed low response rates for both (12.4% and < 0.1%, respectively), which were similar to our study.[28] Several contributing factors were workload of the general practitioners, increasing number of other similar surveys, and no incentives for participating general practitioners. A systematic review identifying strategies to improve response rates on postal and electronic questionnaires demonstrated that the responses were likely almost doubled when there were monetary/non-monetary incentives, recorded delivery, shorter questionnaires, and interesting survey topics.[29] We did not have any funding to provide incentives. Other weakness of the study was Wwe did not clearly define the answer option of 'observation for 48 to 72 hours' in the clinical scenarios. The option for observation for 48 to 72 hours was meant for clinicians who would choose to closely observe the AOM patients without antibiotics treatment for 48 to 72 hours. Without a clear definition, clinicians who would prescribe antibiotics, may also choose observation to see the effect of antibiotics. This would limit us to precisely identify the proportion of clinicians who would solely choose observation by withholding antibiotic treatment. As the last study weakness, this study only covered three adjacent cities in two provinces (Jakarta and a small part of West Java), which certainly does not represent the current management practice of AOM in Indonesia in general (total provinces in Indonesia is 34). To generalize these findings to the rest of Indonesia requires a national scale study that includes multiple cities (rural and urban) representing each province in Indonesia, supported by the *Ministry of Health, Republic of Indonesia.* Given that the self-reported nature of our study is a further limitation, a national study should collect objective data on the actual number of AOM cases (including diagnosis and treatment) from primary care or hospital patient databases. However, our purpose was to survey likely practice and participation in a proposed trial.

There is still a high rate of antibiotic prescribing among Indonesian clinicians for children with AOM. Although it has not been recommended in the guidelines, clinicians would consider using corticosteroids for AOM. Given nearly half of clinicians were interested in participating in a future trial on corticosteroids, our proposed trial is feasible. This survey demonstrated existing gaps in the management of AOM between clinical practice and evidence. It is crucial to translate scientific evidence to clinical practice to improve the quality of the AOM management in children, particularly in Indonesia, by promoting an accurate and affordable diagnostic tool for AOM, such as a pneumatic otoscope, as well as by prescribing antibiotics only for severe AOM, and offering an observation under adequate pain management for mild cases. Therefore, further investigation is required to identify other contributing factors to be able to tackle this problem comprehensively.

Conflicts of Interest

Dr. Ranakusuma (RR) reports grants from The Australian Commonwealth Government, during the conduction of the study.
Dr. McCullough (AMC) reports grants from Advance Queensland Women's Academic Fund - Maternity, an Early Career Researcher award from Bond University, and was named the BUPA Health Foundation Emerging Researcher 2017 during the conduction of the study. She runs two businesses that undertake work outside the submitted work: Not Just Mum and Amanda McCullough Consulting.
Dr. Beller (EMB) reports grants from The Centre for Research Excellence in Minimising Antibiotic Resistance in the Community (CRE-MARC) Grant, National Health & Medical Research Council (NHMRC grants), Australia, during the conduction of the study.

Dr. Del Mar (CDM) reports grants from The Centre for Research Excellence in Minimising Antibiotic Resistance in the Community (CRE-MARC) Grant, NHMRC (Australia) (NHMRC grants), grants from Australian Commission for safety and Quality in Health Care (ACSQHC) (consultancy), personal fees from BUPA (UK) (consultancy), personal fees from Book royalties (Elsevier; Wiley), grants from Cochrane ARI Group, (NHMRC grant), during the conduction of the study.
Dr. Pitoyo (YP), Dr Safitri (EDS), Widyaningsih (WW) have nothing to disclose.

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Availability of data and material

The datasets generated and/or analyzed during the current study are available in the Bond University Research repository, [https://research.bond.edu.au/en/publications/current-management-of-children-with-acute-otitis-media-by-indones].

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Appendix 1. Questionnaires of the study

QUESTIONNAIRE: THE MANAGEMENT OF ACUTE OTITIS MEDIA IN CHILDREN IN DKI JAKARTA, DEPOK, AND BEKASI	
THANK YOU FOR YOUR TIME AND PARTICIPATION IN FILLING THIS QUESTIONNAIRE. PLEASE TICK (V) YOUR ANSWER	
1	**INCIDENCE, ATTITUDE, AND BEHAVIOUR**
1.1	In your personal practice, how many cases of acute otitis media in children did you see in the past 7 (seven) days? Answer: ..... (.....) cases
1.2	Can you estimate the percentage for each age group of AOM patients who came to your personal practice in the past one month? Answer: .....% 0 to ≤ 2 year old .....% 2 to 5 year old .....% ≥ 5 year old
1.3	The diagnosis of acute otitis media was established using tests mentioned below *(you may choose more than one)* Answer: O Clinical history O Visualization of tympanic membrane using a penlight/ headlight O Visualization of tympanic membrane using an otoscope O Visualization of tympanic membrane using a pneumatic otoscope (using Siegel) O Visualization of tympanic membrane using ear endoscope / microscope O Tuning fork O Pure tone audiometry O Tympanometry / impedance audiometry O Tympanocentesis O Others: .....
1.4	What is the most common antibiotic you give for acute otitis media cases in children (*please choose only one of the following*): Answer: O Amoxicillin O Ampicillin O Cefixime O Cefadroxil O Erythromycin O Azitromycin O Amoxicillin-clavulanate O Cotrimoxazole O Others: .....
1.5	What is the most common duration of antibiotics you give for acute otitis media in children Answer: ..... days
2	**CASE SCENARIO (*there is no 'RIGHT' or 'WRONG' answer*)**
2.1	CASE-1 A one-year old boy, accompanied by his mother, came to your practice with a complaint of pain in his left ear for one day. The pain was not severe. He has had a cold for the last two days with a mild fever. At the physical examination, he looked well and alert with temperature 37.8°C. At his ear, nose, and throat examination,

	there was mucous discharge on the nasal cavities, and his throat looked normal. An otoscopic examination showed redness and bulging tympanic membrane of the left ear.	
	Question:	What the best management for the patient above? *(you may choose more than one)*
	Answer:	O Observation for 48 – 72 hours O Decongestant and/or anti-histamine O Antibiotics O Paracetamol O Ibuprofen OR other anti-inflammatory drugs (NSAID) O Corticosteroids O Antibiotic ear drop O Analgesics ear drop O Physical therapy with *(you may have more than one answer)* O Nebulizer O Diathermy O Laser O Others .....
2.2	CASE-2 A four-year old girl, accompanied by her parents, came to your practice with a complaint of pain in her right ear for one day. She has had a cold for the last four days. She had no fever. At the physical examination, the patient looked well and alert. At her ear, nose, and throat examination, there was serous secretion in the nasal cavities and her throat looked normal. An otoscopic examination showed redness and bulging tympanic membrane of the right ear.	
	Question:	What the best management for the patient above? *(you may choose more than one)*
	Answer:	O Observation for 48 – 72 hours O Decongestant and/or anti-histamine O Antibiotics O Paracetamol O Ibuprofen OR other anti-inflammatory drugs (NSAID) O Corticosteroids O Antibiotic ear drop O Analgesics ear drop O Physical therapy with *(you may have more than one answer)* O Nebulizer O Diathermy O Laser O Others .....
2.3	CASE-3 A five-year old girl, accompanied by her parents, came to your practice with a complaint of pain in her right ear for one day, followed by left ear this morning and she had a mild fever. She had experienced acute otitis media in her right ear one month ago. At the physical examination, the patient looked well and alert with temperature 36.8°C. At her ear, nose, and throat examination, there was minimal serous discharge in her nasal cavities and her throat looked normal. An otoscopic examination showed redness and bulging on both tympanic membranes.	
	Question:	What the best management for the patient above? *(you may choose more than one)*

	Answer:	O Observation for 48 – 72 hours O Decongestant and/or anti-histamine O Antibiotics O Paracetamol O Ibuprofen OR other anti-inflammatory drugs (NSAID) O Corticosteroids O Antibiotic ear drop O Analgesics ear drop O Physical therapy with *(you may have more than one answer)* O Nebulizer O Diathermy O Laser O Others .....
3	**BIODATA**	
3.1	Doctor specialty	
	Answer:	O General practitioner O Paediatrician O Otorhinolaryngologist
3.2	Type of practice	
	Answer:	O Primary healthcare O Private or multi doctor clinic O Public hospital O Private hospital
3.3	City of practice	
	Answer:	O DKI Jakarta O Depok O Bekasi O Others: .....
3.4	Age	
	Answer:	O ≤ 30 years old O 31 – 40 years old O 41 – 50 years old O 51 – 60 years old O 61 – 70 years old O > 70 years old
3.5	Gender	
	Answer:	O Male O Female
4	**PARTICIPATION ON THE FUTURE CLINICAL RESEARCH ON THE MANAGEMENT OF ACUTE OTITIS MEDIA IN CHILDREN**	
	Research summary Antibiotic resistance has been emerging as a global public health problem. Antibiotics have been prescribed for almost 50% cases of acute respiratory infections (ARIs) in primary healthcare centres. As those cases are mostly self-limited diseases and caused by viruses, antibiotics have little or no clinical benefits. As part of a common complication of viral ARIs, acute otitis media (AOM) is mostly found in children and is a key reason for antibiotic prescription.	

	Other strategy than antimicrobial treatment is needed due to the progression into recurrent and persistent AOM. Corticosteroids have an important role as an anti-inflammatory agent. A recent study has shown the use of oral corticosteroid as an additional treatment with antibiotics in cases of AOM with discharge through tympanostomy tubes shortened the duration of otorrhea. There are few small trials on the use of corticosteroids also as an additional treatment of antibiotics in AOM in children and the results of its benefits were varied. Therefore, we plan to conduct a large, well-conducted clinical trial in order to assess the effectiveness of corticosteroid for the treatment of AOM in children	
	After reading this summary of the future research of "Corticosteroid as an alternative treatment for acute otitis media in children" that will be held on February 2017 to February 2018, I would be interested to be involved in this future research	
	Answer:	O NO, I am not interested to be involved O YES, I am interested to be involved. Please complete the following questionnaire below.
Thank you for your time in completing the survey *For those who are interested in participating in our future clinical research of "Corticosteroid as an alternative treatment for acute otitis media in children", please complete your contact details and other information below.*		
5	**BIODATA**	
5.1	Name and title	.....
5.2	Home address	....
5.3	Email address	.....
5.4	Telephone no. Mobile no.	....
5.5	Contact preference	O Telephone O SMS / Whatsapp O Email
5.6	Name/type of practice	....
5.7	Working since	..... (month) / ..... (year)
5.8	Practice address	....
5.9	Practice phone no. Fax no.	....
5.10	Is there an otoscope in your practice? Answer:	O Yes O No
5.11	Is there a pneumatic otoscope (with Siegel) in your practice? Answer:	O Yes O No
5.12	Is there a Tympanometer in your practice? Answer:	O Yes O No
5.13	Is there a Pharmacy in your practice? Answer:	O Yes

		O No. Where can your patient can get their medication? .....
6	**CURRENT EMPLOYMENT**	
	Do you work in other practice or institution/organization?	
	Answer:	O No O Yes. Please answer the following questions below
6.1	Name of other practice (primary health centre/clinic/ hospital)	.....
6.1.1	Working since	..... (month) / ..... (year)
6.1.2	Practice address	.....
6.1.3	Practice telephone no. Fax no.	....
6.2	Name of other employment (lecturer/researcher/scientific writer)	.....
6.2.1	Working since	..... (month) / ..... (year)
6.2.2	Address	.....
6.2.3	Practice telephone no. Fax no.	....
7	**EDUCATION**	
7.1	Bachelor	Faculty of Medicine University of ..... City ..... Completion year .....
7.2	Specialist	Faculty of Medicine University of ..... City ..... Completion year .....
7.3	Others (PhD, Masters)	Faculty of ..... University of ..... City ..... Completion year .....
7.4	Internship (PTT)	O No O Yes. Please answer the following questions below: Completion year ..... Duration ..... year
*** ***Thank you for your interest. We will contact you for further information*** ***		

Please return this questionnaire to:
Prof. Dr. dr. SS / dr. RWR
Address: **********
or contact us at +62********

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Original Article

Severe sepsis criteria, PELOD-2, and pSOFA as predictors of mortality in critically ill children with sepsis

Anindita Wulandari, Pudjiastuti, Sri Martuti

Abstract

Background Sepsis is one of the main causes of death in infants and children. Currently, it is defined as a life-threatening organ dysfunction, caused by an inflammatory response of infection. Several organ dysfunction assessment methods are available, but they are not uniformly used.

Objective To compare the accuracy of three mortality predictor tools: severe sepsis criteria, pediatric logistic organ dysfunction (PELOD)-2, and pediatric sequential organ failure assessment (pSOFA), in critically ill children with sepsis.

Methods This prospective cohort study was conducted in the pediatric intensive care unit (PICU) and pediatric high care unit (HCU) of dr. Moewardi Hospital, Surakarta, Central of Java. All patients who met the systemic inflammatory response syndrome (SIRS) criteria were included in our study. The exclusion criteria were congenital anomalies of heart or kidney, malignancy, or hematological abnormalities. The data were taken from laboratory and physical examinations by the physicians on duty. The outcome assessed was mortality.

Results Of 30 subjects, the mean age was 22.22 (SD 29.36) months; the most common infection source was the respiratory tract, followed by gastrointestinal tract and central nervous system. Most subjects were treated in the PICU and had a mean length of stay of 8.70 (SD 11.91) days. Severe sepsis and PELOD-2 were not significant predictors of death. However, pSOFA score was a statistically significant predictor of mortality, with odds ratio 10.11 (95%CI 1.054 to 97.002; P=0.039).

Conclusion Pediatric SOFA (pSOFA) is a better predictor of mortality compared to PELOD-2 and SIRS-severe sepsis. A pSOFA score ≥ 2 increases the risk of mortality by 10.11-fold. **[Paediatr Indones. 2019;59:318-24; doi: http://dx.doi.org/10.14238/pi59.6.2019.318-24]**.

Keywords: *children; mortality; sepsis; PELOD-2; SIRS; SOFA*

Sepsis is one of the leading causes of death in infants and children all over the world.[1-4] Currently, sepsis is defined as multiple organ dysfunction due to an unregulated host response to infection, which affects the course of the disease and prognosis in patients.[5,6] Several scoring systems are used to assess organ dysfunction, which in turn are used to diagnose sepsis. These assessment methods aim to consider the risk of mortality, outcome predictions, prediction of the severity of the disease, and organ failure. Such grading systems also assist clinical decision-making, research standardizing, and comparison of patient services among intensive care units.[5-9]

In 2005, the *International Pediatric Sepsis Consensus Conference* (IPSCC) introduced the SIRS criteria with several additional parameters to assess organ dysfunction in diagnosing severe sepsis,[9] considered the same as those uses to diagnose sepsis nowadays.[6]

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The Indonesian Pediatric Association (IPA) currently recommends PELOD-2 to assess multiple organ dysfunction in sepsis.[8] The latest consensus regarding sepsis in adults (Sepsis-3) uses SOFA to assess organ dysfunction.[6] In pediatric population, Matics *et al.*[10] proposed the pediatric SOFA which has been adapted to age and normal pediatric physiology.

In Indonesia, few studies have compared mortality prediction accuracy in pediatric patients using the new internationally introduced pSOFA criteria. Hence, we aimed to compare several systems for evaluating organ dysfunction (severe sepsis criteria, PELOD-2, and pSOFA) and used to diagnose sepsis in children, with regards to their accuracy in predicting mortality.

Methods

This prospective cohort study was conducted to compare severe sepsis criteria, PELOD-2, and pSOFA in predicting mortality among critically ill pediatric patients with sepsis treated in the PICU and HCU in Dr. Moewardi General Hospital, Surakarta, Central of Java. The study started in September 2018, ended in March 2019, and employed a consecutive sampling method. All pediatric patients diagnosed with sepsis based on the SIRS criteria[9] and whose parents provided written informed consent were included in our study. Patients with previously known malignancy, hematological abnormalities, or congenital heart, lung, or kidney anomalies were excluded from the study. We assessed the variables of severe sepsis criteria, PELOD-2 scores, pSOFA scores, and mortality incidence.

Subjects were assessed by three methods and divided into (+) and (-) groups accordingly. Sepsis is defined as systemic inflammatory response syndrome caused by suspected or proven infection. Subject with sepsis categorized as severe sepsis (-), while subject with sepsis plus one of organ dysfunction as mentioned in **Table 1** was categorized as severe sepsis (+).[9] The PELOD-2 was assessed using two cut-off values, namely ≥ 8 and ≥ 11; PELOD-2 score ≥ 8 was rated as (+) and score < 8 as (-). Similarly, if we used the higher cut-off score, PELOD-2 score ≥ 11 was rated as (+) and score < 11 as (-). Scores for pSOFA were considered (+) for ≥ 2, and (-) for < 2. The mortality outcome assessed and subjects were divided into two groups, namely alive (survivors) and died (non-survivors). Examination and assessment of the dependent variable was performed by the physician in charge of the ICU within the first 24 hours of treatment and was followed until the patient was discharged from the ICU (survived or died).

The description of each study variable was performed for the whole sample according to the distribution of outcome categories (mortality).

Table 1. Organ dysfunction criteria[9]

Cardiovascular dysfunction Despite administration of isotonic intravenous fluid bolus ≥40 mL/kg in 1 hr • Decrease in BP (hypotension) 5th percentile for age or systolic BP <2 SD below normal for age OR • Need for vasoactive drug to maintain BP in normal range (dopamine >5 µg/kg/min or dobutamine, epinephrine, or norepinephrine at any dose) OR • Two of the following: • Unexplained metabolic acidosis: base deficit >5.0 mEq/L • Increased arterial lactate >2 times upper limit of normal • Oliguria: urine output <0.5 mL/kg/hr • Prolonged capillary refill: >5 secs • Core to peripheral temperature gap >3°C
Respiratory • PaO_2/FIO_2 <300 in absence of cyanotic heart disease or preexisting lung disease OR • $PaCO_2$ >65 torr or 20 mm Hg over baseline PaCO2 OR • Proven need or >50% FIO_2 to maintain saturation ≥92% OR • Need for nonelective invasive or noninvasive mechanical ventilation
Neurologic • Glasgow Coma Score ≤11 (57) OR • Acute change in mental status with a decrease in Glasgow Coma Score ≥3 points from abnormal baseline
Hematologic • Platelet count <80,000/mm^3 or a decline of 50% in platelet count from highest value recorded over the past 3 days (for chronic hematology/oncology patients) OR • International normalized ratio >2
Renal • Serum creatinine ≥2 times upper limit of normal for age or 2-fold increase in baseline creatinine
Hepatic • Total bilirubin ≥4 mg/dL (not applicable for newborn) OR • ALT 2 times upper limit of normal for age

BP=blood pressure; ALT=alanine transaminase.

Numerical variables are presented as frequency and percentage (n and %), while categorical variables are presented in mean and standard deviation (mean and SD). The OR value to state mortality risk was calculated and the relationship between each assessment criteria (severe sepsis criteria, PELOD-2, and pSOFA) with mortality was analyzed by Chi-square or Fisher's exact test for the unmet minimum expected value in cross-distribution. Results with P value <0.05 were considered to be statistically significant. The accuracy of predicting mortality for each tool and the comparison of the three tools were also assessed by various diagnostic parameters including sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio (LR +), and negative likelihood ratio (LR -). All data were analyzed with *Microsoft Excel 2007* and *SPSS version 21 for Windows* software.

This study was approved by the Health Research Ethics Committee of the Universitas Sebelas Maret Medical School/Dr. Moewardi Hospital Surakarta.

Results

Of 30 subjects, the mean age was 22.22 (SD 29.36) months. The proportion of males was twice the number of females. The most common infection was respiratory tract infection (22/30). Most subjects (25/30) were treated in the PICU and the rest were in the HCU. The mean of length of stay (LOS) was 8.70 days. The proportion of subjects who died reached 46.7%. The demographic characteristics of subjects are presented in **Table 2**.

In comparing subjects who died to subjects who survived, the group who died had younger mean age (12.61 vs. 30.62 months, respectively), lower mean body weight (6.42 vs.10.33 kg, respectively), and shorter mean length of stay (5.00 vs. 11.94 days, respectively). All subjects who died were treated in the PICU. The characteristics of subjects based on the outcome are shown in **Table 3**.

The three methods to assess organ dysfunction (severe sepsis criteria, PELOD-2, and pSOFA) were analyzed in accordance to mortality incidence (**Table 4**). The PELOD-2 cut-off scores used in this study were ≥ 8 and ≥ 11, while the pSOFA cut-off score was ≥ 2.

Table 2. Subjects' characteristics

Characteristics	N=30
Mean age (SD), months	2.22 (29.36)
Gender, n	
Male	20
Female	10
Mean weight (SD), kg	8.51 (5.62)
Infection, n	
Respiratory tract	22
GI tract	3
CNS	4
Other	1
Hospital unit, n	
PICU	25
HCU	5
Mean LOS (SD), day	8.70 (11.91)
Outcome, n	
Died	14
Survived	16

The significance of prediction accuracy of severe sepsis criteria, PELOD-2, and pSOFA on mortality was analyzed by Fisher's exact test, which revealed that severe sepsis criteria and PELOD-2 were not significant predictors for mortality (P> 0.05). However, the pSOFA was a significant predictor for mortality (P=0.039). The OR value for the pSOFA was also the highest at 10.111 (95%CI 1.054 to 97.002). These results indicate that pSOFA was the best predictor for mortality compared to severe sepsis criteria and PELOD-2 with both cut-off scores of 8 and 11 (**Table 5**).

Table 3. Subjects' characteristics based on outcome

Characteristics	Died (n=14)	Survived (n=16)
Mean age (SD), months	12.61 (16.20)	30.62 (35.74)
Gender, n		
Male	7	13
Female	7	3
Mean weight (SD), kg	6.42 (3.24)	10.33 (SD 6.65)
Infection, n		
Respiratory tract	11	11
GI tract	2	1
CNS	0	4
Others	1	0
Hospital unit, n		
PICU	14	11
HCU	0	5
Mean length of stay (SD), days	5.00 (7.64)	11.94 (14.13)

Table 4. Organ dysfunction assessment system in accordance to mortality incidence

Variables	Total (N=30)	Died (n=14)	Survived (n=16)
Severe sepsis criteria			
Positive	25	13	12
Negative	5	1	4
PELOD-2 (score ≥ 11)			
Positive	6	5	1
Negative	24	9	15
PELOD-2 (score ≥ 8)			
Positive	14	9	5
Negative	16	5	11
pSOFA (score ≥ 2)			
Positive	22	13	9
Negative	8	1	7

Table 5. Statistical analysis and OR value of the relationship between mortality and severe sepsis criteria, PELOD-2, and pSOFA scores

Organ dysfunction assessment system	OR	95%CI	P value
SIRS (severe sepsis)	4.333	0.423 to 44.428	0.336
PELOD-2 (score ≥ 11)	8.333	0.835 to 83.167	0.072
PELOD-2 (score ≥ 8)	3.960	0.865 to 18.119	0.070
pSOFA (score ≥ 2)	10.111	1.054 to 97.002	0.039

The prognostic accuracy of the three methods of assessment for sepsis can be compared with various diagnostic parameters. The highest sensitivity was found using severe sepsis criteria and pSOFA, namely 92.9% for both. The PELOD-2 score ≥ 11 had the highest specificity (93.8%). The diagnostic parameter values for each tool are shown in **Table 6**.

Table 6. The diagnostic parameters of severe sepsis criteria, PELOD-2, and pSOFA as mortality predictors.

Diagnostic parameters	SIRS (severe sepsis)	PELOD-2 (score ≥ 11)	PELOD-2 (score ≥ 8)	pSOFA (score ≥ 2)
Sensitivity	92.9%	35.7%	64.3%	92.9%
Specificity	25.0%	93.8%	68.8%	43.8%
NPV	80.0%	62.5%	68.8%	87.5%
PPV	52.0%	83.3%	64.3%	59.1%
Positive LR	1.238	5.714	2.057	1.651
Negative LR	0.286	0.686	0.519	0.163
Positive PP	52.0%	83.3%	64.3%	59.1%
Negative PP	20.0%	37.5%	31.3%	12.5%

Discussion

In our study, the mean age of subjects who met the SIRS criteria and were treated in the PICU or HCU was 22.22 months (1 year 10 months). The subjects who died had a younger mean age than the survived group (12.61 months *vs.* 30.62 months, respectively). A study in Jakarta stated the same results, which analyzed mortality in pediatric patients with sepsis and found out that the median age of subjects was 15 months (range 2-192) with the highest distribution in the 1 month - 1 year age group (62%).[11] Younger age has been associated with immune system immaturity and is usually accompanied by comorbidities, such as congenital heart disease and kidney disease, thus the incidence of infections that cause sepsis is a factor that increases the risk of mortality.[1,11–13] Also, the *Sepsis Prevalence, Outcomes, and Therapies Study* (SPROUT) conducted in 128 PICUs in 26 countries revealed that patients who met SIRS-severe sepsis criteria based on international consensus had a mean age of 3 years, and the foci of infection were the respiratory system (40%) and bacteremia (19%).[1] In our study, the most common focus of infection was the respiratory system, followed by the central nervous system (CNS) and gastrointestinal (GI) infections. Respiratory tract infection is a major cause of illness and death in infants and children. Environmental factors and highly contagious, air-borne pathogens cause airway infections.[14]

All subjects who died were treated in the PICU with mean LOS of 8.7 days, which was shorter than that of the survived group (11.94 days). This finding was likely due to the fact that PICU patients tend to have more severe clinical conditions than those treated in HCU. Furthermore, patients with severe airway infections generally require invasive breathing support, which is available only in the PICU. In a study validating the PELOD-2 method of the use of mechanical ventilation led to OR of 3.99 (95%CI 2.07 to 7.70) on mortality (P <0.0001).15 A previous study also stated that sepsis increased the risk of death by 18 times compared to non-sepsis patients with infection (OR 18; 95%CI 11 to 28). Most of them died because of sepsis or septic shock (65%), and the course of disease necessitated intensive care unit treatment.[10]

The SIRS criteria (IPSCC 2005) have been used to diagnose sepsis for a long time, but not specific. The components of these criteria were found in >90% of pediatric patients with fever in the emergency room, but in <5% of those who needed intensive care. Tachycardia and tachypnea are among the SIRS criteria often found in mild, non-lethal, viral diseases, thus, the SIRS criteria have a low specificity for identifying the risk of death.[16] In our study, we compared the criteria for severe sepsis with other organ dysfunction criteria and found that PELOD-2 and pSOFA predictive accuracy were better than severe sepsis criteria.

Agyeman *et al.*[17] explained that SIRS conditions with bacteremia increased the risk of death by 1% in the first 30 days, but such risk would increase by 17% if accompanied by organ dysfunction. Hence, the SIRS criteria has been abandoned and replaced by organ dysfunction assessment criteria to assess mortality outcomes in intensive care pediatric patients.

Previous studies have compared SIRS criteria to other sepsis criteria, because the 2005 International Consensus introduced SIRS as a tool for diagnosing sepsis.[18,19] Only a few studies comparing severe sepsis-SIRS criteria due to changes in the definition of sepsis that sepsis is considered equivalent to the diagnosis of severe sepsis according to the old consensus. This current definition was published in sepsis-3.

In our study, the criteria for severe sepsis were not statistically significant (P=0.336) in predicting mortality, although the sensitivity, specificity, NPV, and PPV were 92.9%, 25%, 80.0%, and 52%, respectively. A previous study showed that the criteria for severe sepsis had a 74.5% sensitivity, 42.7% specificity, 96.5% NPV, and 7.4% PPV for predicting mortality. They also noted that the criteria for severe sepsis was not good enough to predict mortality of critically ill children.[19]

We used PELOD-2 score of ≥ 11 based on the consensus guidelines for diagnosis and management of sepsis in children from the *Indonesian Pediatric Association*.[8] We also analyzed the PELOD-2 cut-off score of ≥ 8, since it is commonly used for a comparison in previous studies.[19] To date, there is not a uniform, internationally-recognized, cut-off value for PELOD-2 scores. The initial PELOD-2 validation study found that PELOD-2 scores were significantly higher in non-survivors compared to survivors [mean 14.9 (SD 6.1) *vs.* mean 4.2 (SD 3.2), respectively, P <0.0001].[20] The study showed failure of three organ systems and a mean PELOD-2 score of 7.5 predicted a mortality rate of 7.1%. Whereas failure of three organ

systems and mean PELOD-2 score of 11.5 predicted a mortality rate of 30.5%.[15] A previous study reported that PELOD-2 score of ≥ 8 had sensitivity of 85.0%, while PELOD-2 score of ≥ 11 had sensitivity of 92.6% in the incidence of mortality. Their median PELOD-2 score was significantly higher in the group who died than those who lived (died : survived = 13 : 3; P=0.0001).[20]

We found that PELOD-2 score ≥ 8 lower prognostic value compared to other mortality predictors. Although it was not the best predictor of mortality, PELOD-2 score ≥ 8 was better than severe sepsis criteria based on diagnostic parameters. Both PELOD-2 scores of ≥ 11 and pSOFA scores had advantages in several diagnostic parameters that we analyzed. PELOD-2 score ≥ 11 in our study had the highest specificity and PPV rates of 93.8% and 83.3%, respectively, suggesting the lowest mortality predictor error and highest accuracy of life expectancy, with OR 8.33 (95%CI 0.835 to 83.16). However, this PELOD-2 cut-off was not statistically significant (P=0.072). Schlapbach *et al.*[19] demonstrated good results using PELOD-2 score of ≥ 8 in predicting mortality, with sensitivity, specificity, NPV, and PPV of 88.1%, 55.7%, 97%, and 22.2%, respectively. In addition, study in Indonesia used a PELOD-2 cut-off value of ≥20, and found that such patients had a 7.75 times greater risk of death.[21]

The SOFA, a system for evaluating organ dysfunction, was recommended by the most recent consensus.[6,19] Pediatric SOFA is SOFA with adjustments.[10] The cut-off value in our study was ≥2 as suggested by consensus.[6] Our results showed that pSOFA had advantages in sensitivity, NPV, negative LR, and negative PP than the other tools. The pSOFA was better than other assessment methods, if the aim is to get a more accurate screening result for estimating the risk of death, as pSOFA had the highest odds ratio (OR) value 10.11 (95%CI 1.054 to 97,.002; P<0.05). In contrast, severe sepsis criteria and PELOD-2 scores had a lower risk of mortality and were not statistically significant (P=0.336 and P=0.072, respectively; P> 0.05).

Our result is parallel with that of a previous study which stated that the maximum score of pSOFA (AUC 0.94; 95% CI 0.92 to 0.95) was proportional to PELOD (AUC 0.93; 95% CI 0.91 to 0.95) and PELOD-2 (AUC 0.94; 95% CI 0.92 to 0.95), and better than PMODS (AUC 0.93; 95% CI 0.91 to 0.95) (P<0.001) as a predictor of mortality in patients with sepsis. They also reported that the relationship between pSOFA score on the first day of treatment (AUC 0.88; 95% CI 0.86 to 0.91) and patient mortality was better than the other organ dysfunction assessment systems. It was also proportional to PRISM III (AUC 0.88; 95%CI 0.86 to 0.91). In addition, pSOFA criteria ≥ 2 had an increased risk of death (OR 18; 95%CI 11 to 28), and the most optimal pSOFA cut-off score for predicting mortality was > 8.[10]

A similar study found that pSOFA [(adjusted AUROC 0.892 (range 0.791-0.868)] was statistically significant in assessing mortality outcomes in sepsis patients and even better than PELOD-2 score of ≥8 (AUROC 0.816; 0.777-0.854), qSOFA (AUROC 0.739; 0.695-0.784), and SIRS (AUROC 0.710; 0.664-0.756).[9] Our results suggest that pSOFA and PELOD-2 are better than severe sepsis criteria to predict mortality. This finding may have been related to the examination parameters in pSOFA and PELOD-2, which are better at describing organ function failure than the SIRS method.[19]

Our study had several limitations. The assessment of organ dysfunction was done only once when the patient was admitted to the PICU or HCU. If the patients experienced a worsening of symptoms during the treatment, their scores may have actually gone higher. Also, the study was conducted in two different places (PICU and pediatric HCU), hence, the severity of the disease, environmental factors, and supporting care may have been different. In conclusion, pediatric SOFA (pSOFA) is the best predictor for mortality compared to PELOD-2 and severe sepsis criteria. A positive pSOFA score (pSOFA ≥ 2) increases the risk of death by 10.11 times.

Conflict of interest

None declared.

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Original Article

Chronic kidney disease and emotional-behavioral disorders in adolescents

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Abstract

Background Chronic kidney disease (CKD) is characterized by progressive renal injury with inevitable functional deterioration. This functional loss is usually slow, progressive, and irreversible. Chronic kidney disease profoundly influences the daily routines of pediatric patients and their families, requiring significant psychosocial adaptation by both patients and families.

Objective To assess for potential associations between CKD and emotional/behavioral disorders in adolescents.

Methods This cross-sectional study was done at the Pediatric Nephrology Outpatient Department, Hasan Sadikin Hospital, Bandung, West Java. The consecutive sampling included all patients who fulfilled the following criteria: (1) aged 10-18 years, (2) diagnosed with CKD at least 3 months prior to the study, and (3) whose parents provided informed consent. The Strengths and Difficulties Questionnaire (SDQ) was used to assess emotional/behavioral disorders. Socio-demographic and clinical data were collected from medical records and interviews with parents. Chi-square and Mann-Whitney tests were used in the statistical analyses.

Results A total of 75 subjects with CKD participated in the study. The majority of the subjects were female (53%) and <14 years old (55%). Emotional/behavioral disorders were found in 24 subjects (32%). There were no significant correlations between age, gender, paternal and maternal education level, duration of illness, or treatment with emotional/behavioral problems. However, later stage of CKD was significantly associated with prosocial problems, based on the SDQ assessment.

Conclusion Late stage CKD is significantly associated with prosocial problems of the SDQ scales. **[Paediatr Indones. 2019;59:325-30; doi: http://dx.doi.org/10.14238/pi59.6.2019.325-30]**.

Keywords: *adolescent; chronic kidney disease; mental-emotional and behavioral disorder*

Chronic kidney disease (CKD) is a major, worldwide health problem with increasing incidence and prevalence each year.[1] This disease is characterized by progressive renal injury with inevitable functional deterioration, which is usually slow and irreversible.[2,3]

The epidemiological data on CKD is very difficult to study, as CKD is underdiagnosed and underreported. The prevalence of CKD in children aged 0-15 years in Southeast Asia was reported to be around 329 cases per 1 million children.[2] The Dr. Hasan Sadikin Hospital medical records for the period of 2018-2019 showed that 427 children diagnosed with CKD consulted the Pediatric Nephrology Outpatient Department.

Chronic conditions in adolescence can affect the physical, cognitive, social, and emotional spheres of development, with repercussions for siblings and parents.[4] Chronic kidney disease is a risk factor for psychosocial impairment and psychiatric symptoms. In pediatric population, CKD may negatively impact

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psychosocial development and patient quality of life. Children with CKD often experience significant emotional and behavioral problems, especially those with frequent relapses, steroid dependence, steroid resistance, or limitations in their daily life activities.[5] On a daily basis, they are submitted to dietetic and fluid restrictions, difficult and invasive treatments, and even hospitalizations. However, these negative impacts still remain unrecognized and underestimated.[3,5] A study observed that patients with worse emotional and social performance usually demonstrated a tendency to not follow the medical recommendations. Of transplanted kidney patients, 31% reported that they did not follow medical recommendations and 19% reported previous attempts at self-extermination and/or serious suicidal thoughts.[6]

Chronic kidney disease (CKD) studies in the pediatric population have uncovered an elevated frequency of psychiatric disorders, with the most common being adjustment disorders, depressive symptoms, anxiety, and cognitive impairment. However, the association/correlation between clinical or laboratory features, disease duration and severity and psychological variables remains undetermined.[3]

Optimal management of pediatric CKD should focus on both pharmacological therapy and psychosocial aspects. A multidisciplinary team approach to pediatric CKD is needed, not only to improve clinical outcomes, but also to improve quality of life of these children.[7]

Early identification of emotional and behavior problems is important for early diagnosis and management, in order to improve clinical outcomes and successful transition to adult life. This study aimed to assess for potential associations between CKD factors and emotional/behavioral disorders in adolescents.

Methods

This cross-sectional study was done from July to September 2019 in adolescents with CKD who visited the Pediatric Nephrology Outpatient Department, Hasan Sadikin Hospital, Bandung, West Java, for routine check-ups. The inclusion criteria were patients: (1) aged 10-18 years, (2) diagnosed with CKD at least 3 months prior to the study, and (3) whose parents provided informed consent. Exclusion criteria were patients who had: (1) renal transplantation, (2) a significant life event unrelated to their kidney disease in the past 3 months, such as losing a family member, severe illness of a family member, family structure changes, and (3) previous diagnosis of intellectual disability. Subjects were asked to answer the SDQ.

The *Strengths and Difficulties Questionnaire* (SDQ) was a screening/assessment tool to identify emotional and behavior problems in pediatric populations. The SDQ consisted of 25 items which were divided between 5 scales: (1) emotional symptoms, (2) conduct problems, (3) hyperactivity/inattention, (4) peer relationship problems, and (5) prosocial behavior. A probable SDQ prediction for any given disorder correctly identified 81-91% of the children who definitely had that diagnosis, it had a sensitivity of 85% and a specificity of 80%.[8,9] Socio-demographic and clinical data (age, gender, parental education level, duration of illness, CKD etiology, and stage and therapy of CKD) were obtained by parental interviews and medical records.

We evaluated for possible correlations between CKD and emotional/behavioral problems in adolescents by descriptive statistical and bivariate analyses. Continuous data were reported as mean and standard deviation (SD) when appropriate. Non-parametric variables were compared by Mann-Whitney test. Dichotomous variables were compared by Chi-square test. Analyses were carried out using the *SPSS* statistical package *ver. 22* software. This study was approved by the Research Ethics Committee of Dr. Hasan Sadikin Hospital.

Results

A total of 75 adolescents with CKD were enrolled in the study. The majority of the subjects were female (59%) and aged less than 14 years (52%). The main baseline clinical and socio-demographic characteristics are summarized in **Table 1**.

The majority of parents had secondary-high school educational level (fathers 56%, mothers 52%). The majority of the subjects had CKD duration of illness for less than 3 years (80%), were at stage I (61%), and received conservative therapy (85%).

The characteristics of emotional/behavior disorders are summarized in **Table 2**. Based on the

Table 1. Characteristics of subjects

Characteristics	(N=75)
Age, n (%)	
< 14 years	39 (52)
> 14 years	36 (48)
Gender, n (%)	
Male	31 (41)
Female	44 (59)
Paternal education level, n (%)	
Elementary school	27 (36)
Secondary – High school	42 (56)
Higher education	6 (8)
Maternal education level, n (%)	
Elementary school	30 (40)
Secondary – High school	39 (52)
Higher education	6 (8)
Duration of CKD, n (%)	
< 3 years	60 (80)
> 3 years	15 (20)
Stage of CKD, n (%)	
I	46 (61)
II	8 (11)
III	4 (5)
IV	3 (4)
V	14 (19)
Treatment, n (%)	
Conservative	64 (85)
Renal replacement (HD/PD)*	11 (15)

HD=hemodialysis; PD=peritoneal dialysis

Table 2. Characteristics of emotional/behavior disorders assessed by SDQ in adolescents with CKD

SDQ parameters	Normal	Abnormal
Prosocial, n (%)	72 (96)	3 (4)
Hyperactivity, n (%)	62 (83)	13(17)
Emotional, n (%)	55 (73)	20 (27)
Conduct, n (%)\	61 (81)	14 (19)
Peer relationship, n (%)	65 (87)	10 (13)
SDQ total score interpretation, n(%)	51 (68)	24 (32)

results of SDQ answers, 24 subjects (32%) were identified to have emotional/behavior disorders, with emotional (n=20) and conduct disorders (n=14) having highest prevalences (27% and 19%, respectively). The SDQ total score interpretation were the result of SDQ evaluation, which were evaluated from the SDQ total score of each subject.

Table 3 shows the bivariate analysis results on factors with potential correlations to emotional/behavioral disorders or abnormal SDQ results. In this study, total SDQ score was not correlated with patient

Table 3. Analysis of CKD factors and normal *vs.* abnormal SDQ results

Variables	SDQ			P value
	Normal	Abnormal	Total	
Age, n (%)				0.627
< 14 years	28 (55)	11 (46)	39 (52)	
> 14 years	23 (45)	13 (54)	36 (48)	
Mean age (SD), years	13.5 (2.1)	13.9 (0.1)		0.405*
Gender, n (%)				0.224
Male	24 (47)	7 (29)	31 (41)	
Female	27 (53)	17 (71)	44 (59)	
Paternal education level, n (%)				0.761
Elementary school	17 (33)	10 (42)	27 (36)	
Secondary – High school	30 (59)	2 (50)	42 (56)	
Higher Education	4 (8)	2 (8)	6 (8)	
Maternal education level, n (%)				0.972
Elementary school	20 (39)	10 (42)	30 (40)	
Secondary – High school	27 (53)	12 (50)	39 (52)	
Higher education	4 (8)	2 (8)	6 (8)	
Duration of CKD, n (%)				0.853
< 3 years	40 (78)	20 (83)	60 (80)	
> 3 years	11 (22)	4 (17)	15 (20)	
Mean duration of CKD (SD), months	25.53 (2.48)	26.92 (2.29)		0.716*
Stage of CKD, n (%)				0.296
I	35 (69)	11 (46)	46 (61)	
II	4 (8)	4 (17)	8 (11)	
III	2 (4)	2 (8)	4 (5)	
IV	1 (2)	2 (8)	3 (4)	
V	9 (18)	5 (21)	14 (19)	
Treatment, n (%)				
Conservative (received steroids)	44 (86)	20 (83)	64 (85)	1.000
Renal replacement (HD/PD)	7 (14)	4 (17)	11 (15)	

HD=hemodialysis; PD=peritoneal dialysis, *Mann-Whitney Test

age, as the proportion of subjects with normal SDQ and abnormal SDQ based on age (<14 years *vs.* >14 years) were nearly equal (52% vs. 48%; P=0.627).

We found no significant correlation between gender and emotional/behavior disorders. Both normal SDQ and abnormal SDQ subject majorities were female. There was also no significant correlation between parental educational level and emotional/behavior disorder, as the majority of both parents graduated from secondary high school. In terms of duration of illness, stage of CKD, and treatment modalities, there were no significant correlations between these CKD characteristics and emotional/behavior disorders.

Table 4 shows the bivariate analysis results on factors with potential correlations to the SDQ scales (prosocial, hyperactivity, emotional, conduct, and peer relationship). The only significant correlation revealed was stage of CKD and the prosocial SDQ scale (P=0.011). Adolescents diagnosed with prosocial disorders had mostly stage V CKD or end-stage renal disease.

Table 4. Analysis of CKD factors and SDQ scales

CKD factors	SDQ scales (P values)					SDQ total
	Prosocial	Hyperactivity	Emotional	Conduct	Peer relationship	
Age	0.944	0.288	0.638	0.469	0.066	0.627
Gender	1.000	0.589	0.684	0.302	0.260	0.224
Paternal educational level	0.852	0.543	0.146	0.990	0.902	0.761
Maternal educational level	0.813	0.363	0.924	0.968	0.587	0.972
Duration of CKD	0.883	0.939	1.000	0.208	1.000	0.853
Stage of CKD	0.011*	0.567	0.428	0.542	0.127	0.296
Treatment	0.077	0.609	1.000	1.000	1.000	1.000

*statistically significant (P<0.05)

Discussion

In our study, emotional/behavior disorders were found in 32% of adolescents with CKD. This prevalence was similar to previous studies with mental disorder prevalences ranging from 35.7% to 36.8%.[3] The presence of CKD during adolescence significantly increased the risk of emotional and behavioral disorders.[3] Children with CKD can present with psychological disorders caused not only by the disease itself, but also by the treatment. These children may have limitations in their daily lives, mainly physical ones, due to the characteristics of the disease that require continuous readaptation. In addition, the disease affects not only these children's lives, but also the lives of their families. They also have a tendency to negative self-image and feelings of inferiority in relation to their peers.[10]

Several hypotheses have been suggested to explain this increase in the prevalence of emotional/behavioral disorders. In addition to the stress inherent to CKD and its treatment, studies have pointed out other factors that contribute to the predisposition to psychiatric disorders in this group. Among them are the decrease in levels of brain-derived neurotrophic factor (BDNF) and the low serum level of serotonin in CKD patients.[10,11]

Development refers to the process through which human beings typically grow and mature from infancy through adulthood. The different aspects of development that are measured include gross motor, fine motor, speech, cognitive growth, and social growth. Child development is a complex interaction between brain maturation and the organs involved.[12]

During the preteen, teenage, and young adult years, young people not only undergo dramatic changes in physical appearance, but also rapid changes in physiological, psychological, and social functioning. Hormonally-driven physiological changes and ongoing neurological development occur in the setting of social structures that foster the transition from childhood to adulthood. This period of development comprises adolescence, which has been divided into 3 phases: early, middle and late adolescence.[12,13]

The early adolescence period (10-14 years) is characterized by concrete cognitive thoughts,

egocentricity, and inability to perceive long-term outcomes of current decisions. In the middle adolescence period (15-16 years), cognitive thoughts become more mature (emergence of abstract thought), adolescents may perceive future implications but may not apply them to decision-making, and their strong emotions may drive decision-making. The late adolescence period (17-19 years) is characterized by idealism, a future orientation with a sense of perspective, independent thinking, and increased autonomy.[12,14]

Adolescence is considered to be a difficult period, marked by conflicts in search of one's autonomy, culminating with the redefinition of the individual as one moves towards the introduction of adult life. In patients with CKD, these conflicts are aggravated by the difficulty of managing the disease, feelings of rebellion, and denial of one's physical condition and treatment.10 In our study, the majority of subjects were ≤14 years old (early adolescence) and female. Based on our analysis, age and gender were not associated with emotional/behavior disorders or abnormal SDQ results.

A previous study found a correlation between duration of illness and psychosocial problems in CKD patients. Longer duration of illness was associated with higher psychosocial problem prevalence.[5] On the other hand, our study found that duration of illness was not significantly correlated with emotional/behavior disorders.

Another study showed a probable association between an increase in height, as well as adequate level of hematocrit and albumin, with better quality of life (QoL) in adolescents with CKD. A decrease in the glomerular filtration rate (GFR) was associated with significant impairment of QoL.[15] We found a significant correlation between stage of CKD and prosocial problem based on SDQ results. Stage of CKD was significantly higher among those presenting with prosocial problems.

Children with renal disease who received steroids may be particularly susceptible to the side effects of steroids. Duration of steroid treatment was found to be significantly related to children's anxiety/depression and externalizing problems. This finding may reflect the effect of extended exposure to steroids on the children's physical appearance (body mass index and height). Moreover, it could be hypothesized that anxiety/depression problems may be partly induced by steroid effects on brain regions (i.e., hippocampus and amygdala) involved in mood regulation and in which corticosteroid receptors are densely located.[5,10] In our study, the majority of subjects received steroids and steroid therapy was not significantly correlated with emotional-behavior disorders. The majority subjects in this study were in a state of remission and received low steroid doses.

The limitations of our study were that the screening instrument used was made not for CKD patients, nor did we measure intellectual level (full-scale IQ). Future studies evaluating anthropometric status, laboratory values (hematocrit, albumin, and urea nitrogen level), sexual maturity rate (Tanner stage), as well as duration and dosage of steroids are needed to provide more information on risk factors of emotional- behavior disorders. Longitudinal follow-up of subjects is needed to monitor and evaluate their developmental process.

In conclusion, later stage of CKD has a significant correlation with prosocial problems based on SDQ results. Stage of CKD is significantly higher among those presenting with prosocial problems.

Conflict of interest

None declared.

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Original Article

Heated, humidified high-flow nasal cannula *vs.* nasal CPAP in infants with moderate respiratory distress

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Abstract

Background Respiratory distress is the most common cause of morbidity in premature babies in the delivery room. Nasal continuous positive airway pressure (nCPAP) is widely used as the preferred modality of treatment, although it may cause nasal trauma. Heated, humidified high- flow nasal (HHHFN) cannula is an alternative oxygen therapy, yet the safety and efficacy has not been widely studied.

Objective To compare the safety and efficacy of HHHFN and nCPAP in premature babies with gestational age > 28 to < 35 weeks and moderate respiratory distress.

Methods We conducted a randomized, non-inferiority, clinical trial using HHHFN vs. nCPAP as a treatment for moderate respiratory distress within 72 hours after they had been used. The efficacy endpoints were treatment failure, length of device uses, length of Kangaroo Mother Care (KMC), and full enteral feeding time. Safety assessment included pain score, nasal trauma, and systemic complications.

Results No differences were found in terms of incidence of endotracheal intubation within < 72 hours of HHHFN (20%) compared to nCPAP (18%) (P=0.799). However, there was a significant difference in moderate nasal trauma in nCPAP (14%) compared to HHHFN (0%) (P=0.006). There were no significant differences of blood gas analysis results, full enteral feeding time, length of KMC, length of device uses, and rate of complications (bronchopulmonary dysplasia/BPD, intraventricular hemorrhage/IVH, patent ductus arteriosus/PDA, necrotizing enterocolitis/NEC and late onset neonatal sepsis/LONS) between the nCPAP and HHHFN groups.

Conclusion The HHHFN is not inferior to nCPAP in terms of the safety and efficacy as primary non-invasive therapy in premature babies of gestational age > 28 to < 35 weeks with moderate respiratory distress . Compared to nCPAP, HHHFN induced lower nasal trauma. **[Paediatr Indones. 2019;59:331-9; doi: http://dx.doi.org/10.14238/pi59.6.2019.331-9]**.

Keywords: *premature; nCPAP; HHHFN; respiratory distress; non-inferiority trial*

Respiratory distress in neonates should be handled with care and as early as possible to prevent further complications or even death. Neonatal stabilization consists of preventing hypothermia, airway management, supporting optimal respiration and adequate circulation, as well as preventing hypoglycemia, which should be performed in all cases of respiratory distress, regardless of their etiology.[1] In general, non-invasive or invasive ventilation therapy is given to prevent respiratory failure. Endotracheal intubation is the limit of invasive ventilation therapy.[2]

Based on animal studies, invasive ventilation therapy, even for short durations, was associated with decreased lung function, which induced alveolar inflammation and surfactant deactivation, as well as repression of alveolar growth. Excessive tidal volume (more than 6 kg/body weight) leads to ventilator-induced lung injury (VILI). Atelectrauma, which is alveolar trauma associated with less than optimal airway opening, induces the production of biochemical

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mediators that lead to inflammation of lung tissue (biotrauma). Ventilator-induced lung injury is closely related to increased incidence of bronchopulmonary dysplasia.[3]

Currently, nasal continuous positive airway pressure (nCPAP) is the first choice of non-invasive ventilation therapy in infants with respiratory distress. Nasal CPAP is used for both primary therapy, such as in respiratory distress syndrome, obstructive apnea, neonatal pneumonia, and meconium aspiration syndrome, as well as secondary therapy, such as in post-extubation from a mechanical ventilator. Studies have shown the benefits of nCPAP to be alveoli recruitment, preventing alveolar collapse, decreasing lung resistance, maintaining an open airway, as well as increasing lung residual capacity, transpulmonary pressure, and lung compliance. Despite its many advantages, the application of nCPAP is not always easy, with problems varying from nCPAP dislodgement, nasal trauma, and infant discomfort to difficulty applying Kangaroo Mother Care.[4,5]

Many neonatal intensive care units now use heated, humidified high-flow nasal (HHHFN) cannula for respiratory distress in newborns. Despite the limited studies about its safety and efficacy, HHHFN continues to gain popularity among neonatologists. Clinical trials have provided evidence for its effectivity as both primary and secondary therapy, while others have conflicting results. HHHFN is a method to provide respiratory support using a high-speed (> 2 L/min), warmed (37°C), and humidified (100% relative humidity or containing H_2O 44 mg/L) airflow through a nasal cannula. Some advantages of HHHFN are improved lung compliance and alveolar-capillary gas fraction exchange, as well as reduced upper airway dead space, airway resistance, burden of body metabolism in respiratory air conditioning function, in addition to decreased work of breathing and positive airway pressure creation for lung recruitment.[6-9]

A Cochrane's meta-analysis concluded that more clinical studies with good design are needed in order to provide evidence for the effectivity and efficacy of HHHFN compared to nCPAP, as initial treatment of respiratory distress in infants.[7] Such study should be done, not only in developed countries, but also in developing countries, especially in densely-populated nations with high newborn mortality rates and low antenatal steroid coverage. Therefore, we aimed to conduct a clinical study to compare the safety and efficacy of HHHFN to nCPAP as a primary therapy modality in preterm infants.

Methods

This study was a non-inferiority, randomized, clinical trial to determine treatment failure/success within 72 hours of treatment between HHHFN and nCPAP in the management of moderate respiratory distress in preterm newborns. It was undertaken at the national referral neonatal intensive care unit (NICU) at Cipto Mangunkusumo Hospital from June to September 2017.

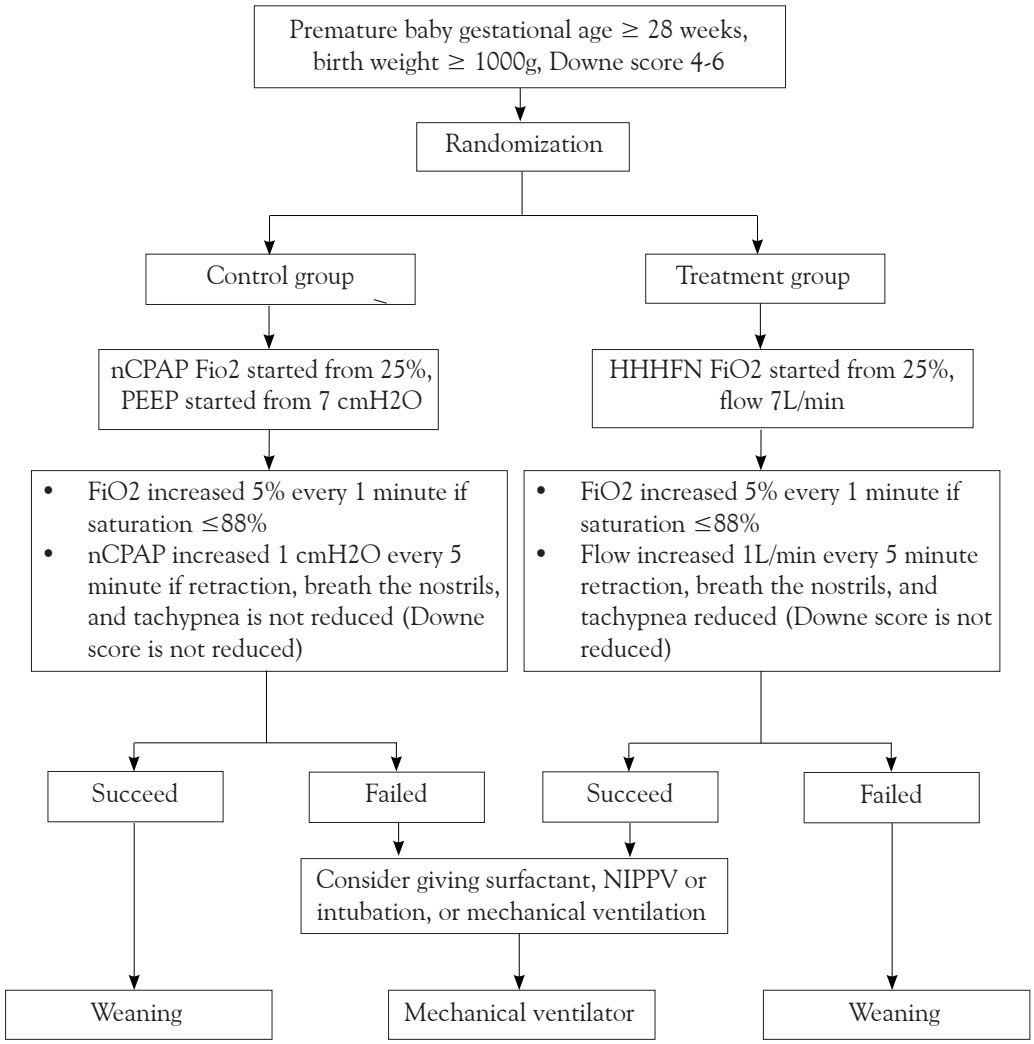
Subjects who met the inclusion criteria were allocated with a computerized, four-block, randomization technique to the treatment group, HHHFN, or the control group, nCPAP. The inclusion criteria were neonates with gestational age ≥ 28 to < 35 weeks, birth weight ≥ 1,000 grams, moderate respiratory distress (Downe's score < 6) from birth or < 24 hours of age, and had never received prior non-invasive ventilation support. The exclusion criteria were severe respiratory distress (Downe's score ≥ 6), recurrent apnea > 2 times in 1 hour, respiratory distress due to problems outside the lungs, congenital anomalies that aggravated the respiratory distress, contraindication of using non-invasive ventilation (e.g., hernia diafragmatica), congenital metabolism abnormalities, and requiring surfactant therapy. This study was approved by the Research Ethics Committee of the University of Indonesia Medical School and the Research Ethics Committee of Cipto Mangunkusumo Hospital.

Gestational age was determined by the New Ballard score.[10] The degree of respiratory distress was quantified by Downe's score.[11,12] The control group was supported with non-invasive ventilation of nCPAP using a BC 161 Bubble CPAP system (*Fisher Paykel*®), while the infants in the treatment group received HHHFN using an optiflow premature system (*Fisher Paykel*®). Blood gas analysis was measured using *pHOx Ultra*® (Nova Biomedical) machine. Peripheral saturation was read by radical 7 pulse oximetry (*Massimo*®). Pain score was quantified using the Cipto Mangunkusumo Hospital neonatal pain monitoring score adopted from the Neonatal Pain

Assessment tool.[13] Nasal trauma due to the nasal prong was assessed by Fisher score.[13] The duration of full enteral feeding time was measured in hours, with rounding up to 1 day if ≥ 12 hours. Both HHHFN and nCPAP were applied as early as respiratory distress detected, whether in delivery room or in neonatal care unit. Failure was defined when the baby got intubated less than 72 hours of treatment, while success was defined when the baby has never been intubated. The study protocol flow chart is shown in **Figure 1**.

Results

This study was conducted from June to September 2017. A total of 169 babies were born with gestational age ≥ 28 to < 35 weeks in Cipto Mangunkusumo Hospital, 100 of whom met the inclusion criteria. The study flow chart with outcomes is shown in **Figure 2**. The post natal maternal and infant characteristics data were not significantly different between the nCPAP and HHHFN groups (**Table 1**).



Note: NIPPV=non-invasive positive pressure ventilation

Figure 1. A flow diagram of study recruitment

Table 1. Characteristics of maternal and infant data

	Characteristics	nCPAP (n=50)	HHHFN (n=50)
Maternal	Chorioamnionitis, n (%)		
	Yes	2 (4)	1 (2)
	No	48 (96)	49 (98)
	Antenatal care, n (%)		
	Regular	44 (88)	47 (94)
	Irregular	6 (88)	3 (94)
	Premature rupture of membranes, n (%)		
	< 18 hours	31 (62)	29 (58)
	≥ 18 hours	19 (38)	21 (42)
	Urinary tract infection, n (%)		
	Yes	2 (4)	2 (4)
	No	48 (96)	48 (96)
	Hypertension during pregnancy, n (%)		
	Yes	13 (26)	14 (28)
	No	37 (74)	36 (72)
	Hyperglycemia during pregnancy, n (%)		
	Yes	2 (4)	0 (0)
	No	48 (96)	50 (100)
	Antenatal steroids, n (%)		
	Incomplete	33 (66)	35 (70)
	Complete	7 (34)	15 (30)
	Antenatal hemorrhage, n (%)		
	Yes	2 (4)	6 (12)
	No	48 (96)	46 (88)
Infant	Gender, n (%)		
	Male	22 (44)	23 (46)
	Female	28 (56)	27 (54)
	Median gestational age (range), weeks	33 (28-34)	33 (28-34)
	Median birth weight (range), grams	1,695 (1,010-2,735)	1,710 (1,002-2,600)
	Type of delivery, n (%)		
	Vaginal	12 (24)	15 (30)
	Caesarian section	38 (76)	35 (70)
	History of resuscitation, n (%)		
	None	12 (24)	14 (28)
	Early nCPAP, HHHFN	31 (62)	28 (56)
	VTP without intubation	5 (10)	8 (16)
	Crystalloid support	2 (4)	0 (0)
	Chest compressions and adrenaline	0 (0)	0 (0)
	Median Downe score (range)	5 (4-6)	5 (4-6)
	Radiology diagnosis, n (%)		
	TTN or RDS grade 1-2	44 (88)	44 (88)
	RDS grade 3-4, MAS, pneumonia, others	6 (12)	6 (12)
	Early-onset neonatal sepsis, n (%)	1 (2)	3 (6)

TTN=transient tachypnea of the newborn, MAS=meconium aspiration syndrome

There was no significant difference related to the treatment failure between HHHFN and nCPAP, blood gas analysis results, length of full enteral feeding and KMC (Tables 2, 3, 4). Moderate nasal trauma was found more frequent in nCPAP group compared to HHHFN group (P=0.006).

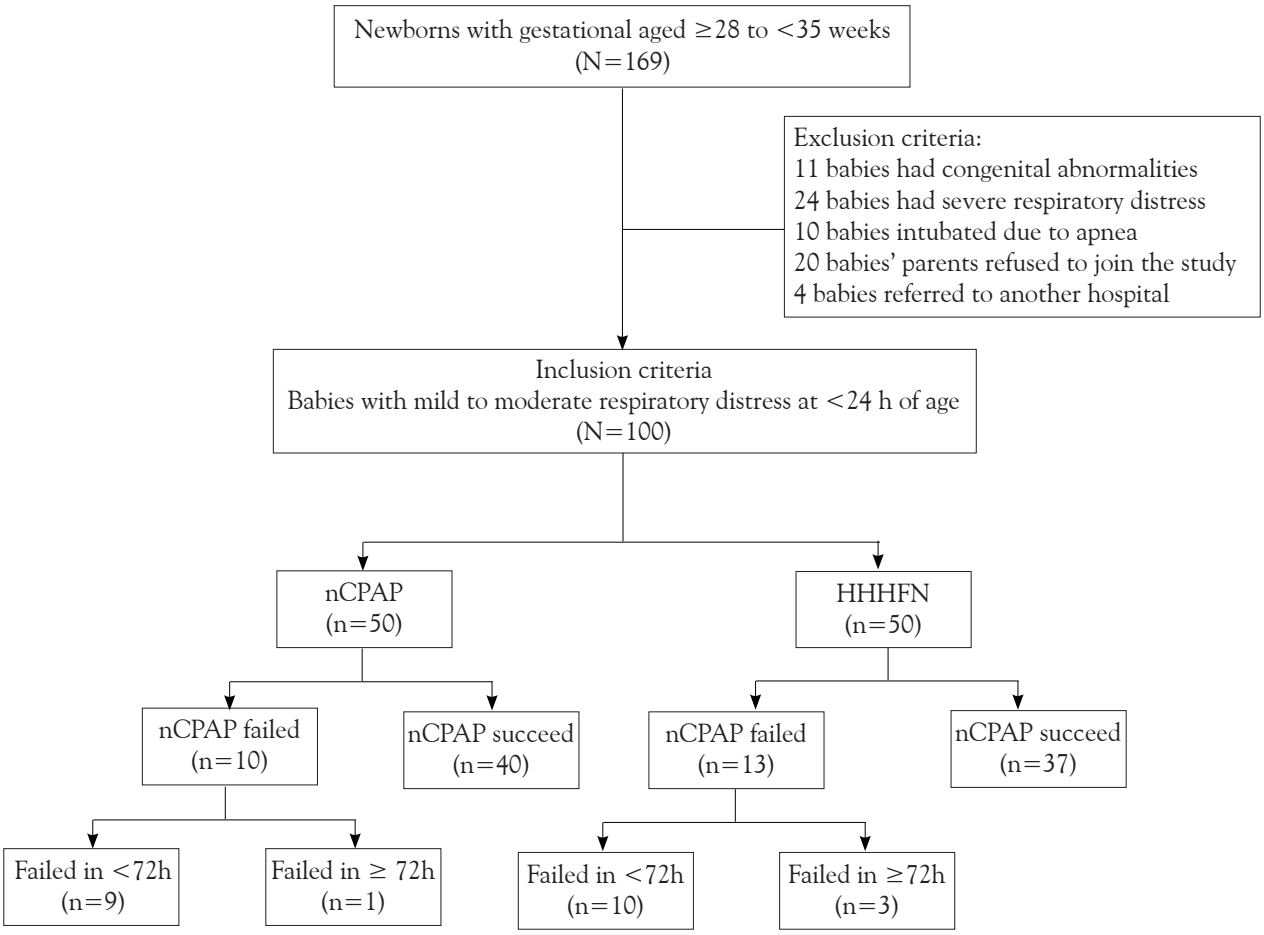


Figure 2. Study outcomes flow chart

Table 2. Comparison of failure rates and nasal trauma between the nCPAP and HHHFN groups

			nCPAP (n=50)	HHHFN (n=50)	P value
Treatment failure	Failed ≤72 hours, n(%)	No	41 (82)	40 (80)	0.799*
		Yes	9 (18)	10 (20)	
	Failed >72 hours, n(%)	No	49 (88)	47 (94)	0.309*
		Yes	1 (2)	3 (6)	
Nasal trauma at 3 days post-treatment	None or grade 1		43 (86)	50 (100)	0.006*
	Grade 2 or 3		7 (14)	0	

Table 3. Comparison of pH, pCO2, and arterial blood pO2 levels between nCPAP and HHHFN groups

	Mean nCPAP (SD)	Mean HHHFN (SD)	Mean difference (95%CI)	P value
pH	7.42 (0.10)	7.40 (0.09)	0.02 (-0.16 to 0.59)	0.259*
pCO_2, mmHg	37.65 (12.98)	41.37 (11.82)	-3.72 (-8.65 to 1.20)	0.137*
pO_2, mmHg	66.98 (30.69)	72.80 (37.46)	-5.81 (-19.41 to 7.78)	0.398*

Table 4. Comparison of length of device usage, mean pain score, length of full enteral feeding time, and length of KMC between the nCPAP and HHHFN groups

	Median nCPAP (range)	Median HHHFN (range)	P value
Length of device usage, hours	25 (1-425)	27 (1-644)	0.537*
Mean pain score	2.33 (1.33-5.66)	2.33 (1.33-6.00)	0.502*
Time to full enteral feeding, days	6 (1-24)	7 (1-28)	0.959*
Length of KMC, hours	0 (0-25)	0 (0-28)	0.724*

Discussion

To our knowledge, this is the first study to compare the safety and efficacy of HHHFN and nCPAP as primary therapy while still in the delivery room. It is our standard to give nCPAP to babies who experience respiratory distress using cold and dry air via t-piece rescucitaor and single nasal prong. In our study we tried to give HHHFN as early as possible while still in the delivery room based on the idea that HHHFN could also provide positive airway pressure compared to nCPAP if given with an appropriate gas flow.[13,14]

Contrary to other studies that started HHHFN at different flow rates (Yoder *et al.* 3-5 L/min,[8] Iranpour *et al.* 1.5-3L/min,[14] Roberts *et al.* 6-8 L/min,[15] Shin *et al*. 3-7 L/min,[16] Ciufini *et al.* 4-6 L/min,[17]), we started HHHFN at 7 L/min. We expected that creating greater positive airway pressure would be more helpful for the neonates to pass the transition period from the time they were in the delivery room.[13,14]

Our subjects had a greater failure proportion in both the nCPAP and HHHFN groups compared to studies done in developed countries (USA, Australia and Norway).[15,17,18] There were 68 babies whose mothers did not complete steroid antenatal course, and 32 babies completed steroid antenatal course. Failure happened in 25% and 18% babies whose mother had not completed and completed antenatal steroid course, accordingly. This higher failure rate may have been due to incomplete or lack of antenatal steroid administration that 92.3% of subjects experienced respiratory distress caused by transient tachypnea of the newborn or grade 1 respiratory distress syndrome, both of which tend to improve rapidly. There were 74 babies who got HHHFN or nCPAP early in the delivery room, while 26 babies got late respiratory distress and started to get HHHFN or nCPAP in neonatal care unit. There was 25% failure rate for babies who got respiratory support early in the delivery room, and 15% failure rate for babies who got late respiratory support in neonatal care unit.

Our results were in agreement with other studies in that preterm babies had shorter treatment duration with nCPAP than with HHHFN, yet the difference was not statistically significant.[8,15,16] This result might have been due to nCPAP having more stable (non-fluctuative) positive pressure airflow compared to HHHFN.[13,14,18] In fact, from its conception, HHHFN was not intended to deliver positive pressure, but high continuous airflow, which eventually creates airway pressure whenever there is airway resistance.[18]

The benefit of using HHHFN is that the continuous airflow can reduce the respiratory loss space, such that ventilation becomes more effective. Upon infant inspiration, if the inspiratory airflow is lower than HHHFN airflow, additional airflow will enter the airway. On the contrary, during expiration, the difference in directions of expiratory airflow to the HHHFN continuous flow creates airway pressure opening.[4,9,18]

There were no significant differences between pH, pCO_2, and pO2 at 30 to 60 minutes post-nCPAP usage compared to HHHFN. This finding indicates that airway pressure and ventilation were comparable between the nCPAP and HHHFN groups. Because partial pressure of arterial blood oxygen is strongly influenced by partial pressure of alveolar oxygen,[2,19,20,23,24] degree of alveolar expansion is strongly influenced by positive airway pressure, and there were no significant differences in FiO_2 usage between the two groups on blood gas analysis, we can conclude that the positive airway pressure is comparable in both devices.

The partial pressure of carbon dioxide in blood is determined by degree of CO_2 exhaustion, known as minute ventilation. Tidal volume and respiratory rate are the two determining factors of minute ventilation. If the respiratory rate is assumed to be comparable

between groups (comparable tachypnea determinant factors such as Downes score, birth weight, gestational age, and pain score), then the tidal volumes in both research groups were comparable. With regards to similar birth weight, gestational age, and thorax x-ray, we can conclude that positive airway pressure created comparable tidal volumes in both groups.

There were no significant differences in median duration of nCPAP and HHHFN usage [25 (range 0-425) hours and 27 (range 0-644) hours, respectively]. These durations were shorter than the median (interquartile) reported by Manley *et al.* for nCPAP [48 (48-168) hours] and for HHHFN [72 (48-144) hours].[18] These differences were probably caused by the older gestational age (median 33 weeks) of our subjects compared to theirs (median 32 weeks).[18] Another possibility was our limiting subject inclusion to only preterm babies with moderate respiratory distress. As such, 60% of the etiology of subjects' respiratory distress was TTN or 1st to 2nd degree hyaline membrane disease (HMD), which naturally get better faster.[16,18]

We measured pain score in the first three days on the premise that most non-invasive airway support will be stopped in less than 72 hours. We suspected that the degree of pain was correlated to nCPAP and HHHFN application. There were no differences in median pain score between the nCPAP or HHHFN groups. These findings might have been due to nursing skill improvements in conducting a neonatal comfort program, such as pain score measuring as a vital sign, early intervention if subject experience painful stimuli, newborn nest usage, midline position in babies, hydroxycoloid tape to prevent blisters, minimal handling program, and giving sucrose and/or pacifier for non-nutritive sucking.[21,22] Similarly, Klingenberg et al. compared pain scores in babies given nCPAP and HHHFN and found that mean cumulative Edin scores were 10.7 and 11.1, respectively; (P=0.25).[23]

In our study, we measured the time to full enteral feeding, not full oral feeding, for study feasibility, and found no significant difference between the two groups [6 (range 1-24) *vs.* 7 (1-28) days; (P=0.959)]. Shin *et al.* in Korea also reported median (interquartile) achievement of full enteral feeding in babies > 30 weeks with nCPAP or HHHFN were 6 (5-9.5) days and 6 (5-9) days, respectively.[16] This observation was likely due to the application of an aggressive enteral nutrition program soon after infants are stable in our unit.[24] With dyspnea quickly resolved (nCPAP and HHHFN median usage of 25 and 27 hours, respectively), the sooner the infant's condition stabilizes, hence, the sooner the infant achieves enteral feeding.[24,25] This program manages to overcome factors that delay reaching full enteral feeding, such as hesitation due to aspiration risk in increasing feeding volume as long as the babies are still in positive airway pressure support, increased abdominal circumference, and vomiting.[26]

Subjects' median weight was 1,800 grams and median gestational age was 33 weeks. Such infants will benefit from Kangaroo Mother Care (KMC). However, 80% of our subjects' mothers did not do KMC. Low KMC duration in babies undergoing nCPAP or HHHFN may be caused by mother's lack of awareness of the importance of intermittent KMC, limited supporting facilities such as KMC chair, inexpensive and comfortable accommodation for mothers in the hospital area, and poor road conditions that make it difficult for the mother to make frequent trips to the hospital. Unfortunately, we did not measure the duration of desaturation episodes during KMC. Nor did we analyze KMC duration post-nCPAP or post-HHHFN, due to the limited observation period and the fact that it was a peripheral issue to our study aim.

Although our hospital used hydroxycoloid tape (*Duoderm Extra-Thin®*) around subjects' noses to prevent nasal trauma during nCPAP, 7 babies (14%) from the nCPAP group experienced 2nd degree nasal trauma. In contrast, no babies had nasal trauma in the HHHFN group. Similarly, a study compared nasal trauma degree between nCPAP and HHHFN usage and reported significantly higher mean (SD) nasal trauma degree with nCPAP [11.7 (10.4)] than with HHHFN [2.8 (5.7)]; (P < 0.001).[27] All 7 infants with 2nd degree nasal trauma were < 32 weeks gestational age, had birth weight between 1,020 to 1,210 grams, and were in the nCPAP group. Based on our observation, the large size of the nasal prong and inability to switch to HHHFN (due to study protocol) were the main causes of the 2nd degree nasal trauma problem.

Warm and humid air helps maintain optimal infant skin integrity. Chang *et al.* noted in an in-vitro study that there were significant mean (SD)

differences in warm air in HHHFN [83 (3.1)%] compared to nCPAP [76 (0.81)%], with 3-8 L/minute air flow. This might have been another factor in preventing nasal trauma.

Fifteen percent was considered to be the significant difference in this non-inferiority study. Ideally, the largest percentage difference for a non-inferiority study is < 10%. However, if we apply this principle to our study, we would have needed 350-400 subjects. Another multicenter, non-inferiority clinical study is needed to acquire more subjects. However, there was congruence from the 9 outcomes in our study, indicating that efficacy and safety OF HHHFN and nCPAP therapy were not that different in infants of gestational age > 28 to < 35 weeks with moderate respiratory distress.

Intra-tracheal pressure measurement is the gold standard in comparing positive airway pressure between nCPAP and HHHFN. Unfortunately we could not conduct this test due to limited equipment, facility, and cost. However, this study is the first to compare HHHFN and nCPAP as primary therapy from the delivery room with outcomes (pH, pCO_2, pO_2, duration of ventilation support, and time until full enteral feeding) that may serve as surrogates for intra-tracheal pressure measurements.

In conclusion, in newborns with gestational age ≥ 28 to < 35 weeks and birth weight ≥ 1,000 grams with moderate respiratory distress syndrome aged less than 24 hours, there are no significant differences in failure of therapy at 72 hours post-device usage, pCO_2, pO_2, mean blood pH level in 30 minutes to 1 hour post-device usage, duration of device usage, time until full enteral feeding, or in duration of KMC between those who received nCPAP compared to HHHFN. But, there is a significant difference related to nasal trauma of 2nd or 3rd degree post-72 hours of usage between the 2 groups.

From these findings, we suggest that heated, humidified high-flow nasal cannula can be an alternative, non-invasive positive airway therapy in infants ≥ 28 to < 35 weeks gestational age with moderate respiratory distress, and aged less than 24 hours. Similar multicenter studies are needed with a larger sample size and infants with gestational age ≤ 28 weeks. Both HHHFN and nCPAP are better if warmed (36.5-37.5°C) and humid (containing water vapor 44 mg/L) gas sources are used.

Conflict of interest

None declared.

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Original Article

Lung ultrasound in diagnosing neonatal respiratory distress syndrome: a meta-analysis

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Abstract

Background Neonatal respiratory distress syndrome (NRDS) is commonly diagnosed by clinical sign and symptoms, blood gas analysis, and chest x-ray. In the past, lung ultrasound (LUS) was not standard for NRDS examination. Many studies show that ultrasound diagnostic tool for NRDS is accurate, reliable, low cost, easy to use, and safe because due to no ionizing radiation.

Objective To determine the sensitivity and specificity of LUS in diagnosing NRDS.

Methods This meta-analysis study was conducted LUS as a diagnostic tool for NRDS. Inclusion criteria were all studies from PubMed, Embase, and The Cochrane Library, without any limitation on published journals, as well as using keywords or search terms of ultrasound, neonatal, and respiratory distress syndrome. Statistical analysis was undertaken using *MedCalc®* version 18.2 software.

Results Seven studies with a total of 580 patients met the inclusion criteria. Proportional meta-analysis obtained random effects models, with total sensitivity of LUS was 97.2% (95% CI for I2 74.24 to 92.88; P<0.0001) and specificity of LUS was 94.8% (95% CI for I2 88.60 to 98.03; P<0.00001).

Conclusion Lung ultrasound should be considered as a diagnostic tool for NRDS because it is high in sensitivity and specificity, inexpensive, safe, as well as limited radiation exposure. **[Paediatr Indones. 2019;59:340-8; doi: http://dx.doi.org/10.14238/pi59.6.2019.340-8]**.

Keywords: *neonatal respiratory distress syndrome; lung ultrasound*

The capability of newborn babies to adapt to life outside the uterus is important for their survival.[1] After birth, almost all body function are switched from fetal to newborn. Respiratory system, in particular, has a new important role in infant survival.[2] Some lung disorders do not affect fetus inside uterus because all nutrition and oxygen from the mother through a placenta.[3] Neonatal respiratory distress syndrome (NRDS) is a primary cause of morbidity and mortality in preterm newborn because of breathing disorders involving a lack of surfactant in the lung and structural pulmonary immaturity.[4,5]

Neonatal respiratory distress syndrome is a common reason for admission to the neonatal intensive care unit (NICU), with newborns exhibiting tachypnea, poor feeding, nasal flaring, grunting,

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cyanosis, intercostal retraction, and reduction of respiratory sounds upon lung auscultation. In under developed countries, the mortality rate of NRDS is ten times higher than that of in developing countries, reaching about 60%.[6,7]

A study reported that gestational age, intrauterine distress, and gestational diabetes could increase the risk of NRDS.[8] Full term newborns have a lower risk than preterm newborns. Neonatal respiratory distress syndrome is considered as a neonatal emergency, with reported prevalence of around 47.5% in Cameroon,[9] 23% in Karachi,[10] 26.2% in Nigeria,[11] 12% in USA,[12] and 9-14% in Indonesia.[13]

Neonatal respiratory distress syndrome is generally diagnosed by clinical signs and symptoms, blood gas analysis, and chest x-ray (CXR). Chest x-ray is a routine examination to evaluate lung and other chest anomalies in neonates. It may be required more than once. A study using thermoluminescence dosimetry showed that the total risk of radiation to the baby was low for one time CXR.[14] In addition, another study found that neonates, including preterm newborns were exposed to 65-67 microGy in evaluations using entrance skin dose (ESD) measurements.[15] Although CXR is deemed safe, previous studies showed that cancer risk is inversely proportional with age, suggesting that neonatal radiosensitivity is higher than that of children or adults, especially in neonates who are exposed to more than 70 microGy.[16]

The latest studies have shown that lung ultrasound has high sensitivity and specificity as a diagnostic tool for NRDS.[17] In the past, lung ultrasound was not a standard examination tools for NRDS examination. However, many studies showed that ultrasound is a useful diagnostic tool due to its good accuracy, reliability, low cost, ease of use, and safety because it has no ionizing radiation.[18,19] As such, we conducted this study to determine the sensitivity and specificity of lung ultrasound in neonatal respiratory distress syndrome.

Methods

This proportional meta-analysis study was performed by collecting data from the latest studies about sensitivity and specificity of lung ultrasound as a diagnostic tool for NRDS. Studies were collected and identified in August 2018 using databases of PubMed 2000-2018, Embase 2000-2018, and The Cochrane Library 2008-2018. Search terms were neonates, lung, ultrasound, and respiratory distress syndrome.

The study subjects were the total number of fulfilling the inclusion criteria namely randomized control trials, case-control studies, or prospective studies; neonates of ≤ 42 weeks gestational age; newborns aged 0-28 days; neonates suffering from respiratory distress syndrome diagnosed with using clinical signs and chest x-ray; and full text manuscript. The exclusion criteria consisted of in silico, in vitro, in vivo, or ex vivo experimental animal studies; lung ultrasound used as diagnostic tool for diagnosing other than NRDS; lung ultrasound used as a diagnostic tool for NRDS in children other than neonates; neonates congenital heart disease; studies lacking of sensitivity and specificity data; studies lacking of full text manuscripts; studies found more than once in other websites or databases; and meta-analysis studies.

Studies were assessed for selecting and reporting bias. Quality was assessed using the *Quality Assessment of Diagnostic Accuracy Studies 2* tool. It was accessed in *Review Manager 5.3* software which has four domains for risk of bias and applicability concerns: patient selection, index test, reference standard, and test flow and timing. Five team members individually scored each study for all domains.

Patient selection was rated to be low risk if neonates were suspected for having respiratory distress syndrome. Applicability concerns were rated to be low risk if neonatal congenital heart disease had been excluded. The index test was rated to have low risk if sonographers were blinded to the chest x-ray results. Applicability concerns were about specification and capability of ultrasonography machine. The reference standard was rated to be low risk if the clinicians were blinded to the lung ultrasound results. Applicability concerns were about good clinical signs and tests. Flow and timing test was rated low if neonates underwent the same clinical examinations and chest x-ray, with interval time between chest x-ray and lung ultrasound was less than 24 hours. Differences in opinion among team members were resolved by discussion.

In our study, a 95% confidence interval indicates a 95% probability that the sample is representative mean of real population. The inconsistency (I^2)

test was used to quantify heterogeneity, and it was considered to be significant when greater than 50%. Sensitivity is a true positive rate which measures the proportion of real positives. Specificity is a true negative rate which measures the proportion of real negatives.

We performed statistical analyses using *MedCalc®* version 18 software, with a proportional meta-analysis. We assessed statistical heterogeneity using the I2 statistic, indicating significance if the I2 was greater than 50%. We reported the 95% confidence interval (CI) on all estimates and used a random-effects model for meta-analysis due to heterogeneity.

Results

Database search in August 2018 with keywords such as 'lung ultrasound,' 'neonatal,' and 'respiratory distress syndrome' yielded 649 PubMed studies, 97 Embase studies, and 1 Cochrane Library study (**Figure 1**). Of these, 7 studies with a total of 580 patients met our inclusion criteria. Those studies were fulfilled to analyze (**Table 1**).

Most studies were from PubMed, as it is a database connected to many libraries, scientific studies, and articles from all over the world. Some of studies from Embase and The Cochrane Library were also found in PubMed. The oldest article included was published in 2006 by Bober *et al.*[19] The most recent study was written by El-Malah *et al.*[20] and published in 2015. The largest sample size was found in Bober *et al.*[19] (131 subjects) and the smallest sample size was found in Lovrenski[21] study (47 subjects). Two studies were done in Italy while 5 others were conducted in India, Poland, China, Serbia, and Egypt. Those 7 studies comprised of 5 prospective studies and 2 case-control studies (**Table 1**).

The studies evaluated diagnostic methods, in terms of lung ultrasound operator, technique, equipment, and diagnostic criteria, as seen in **Table 2**. The proportional metaanalysis revealed a total LUS sensitivity of 97.2% (95% CI for I2 74.2 to 92.8; P<0.0001) and LUS specificity of 94.8% (95%CI for I2 88.6 to 98.0 ; P<0.0001).

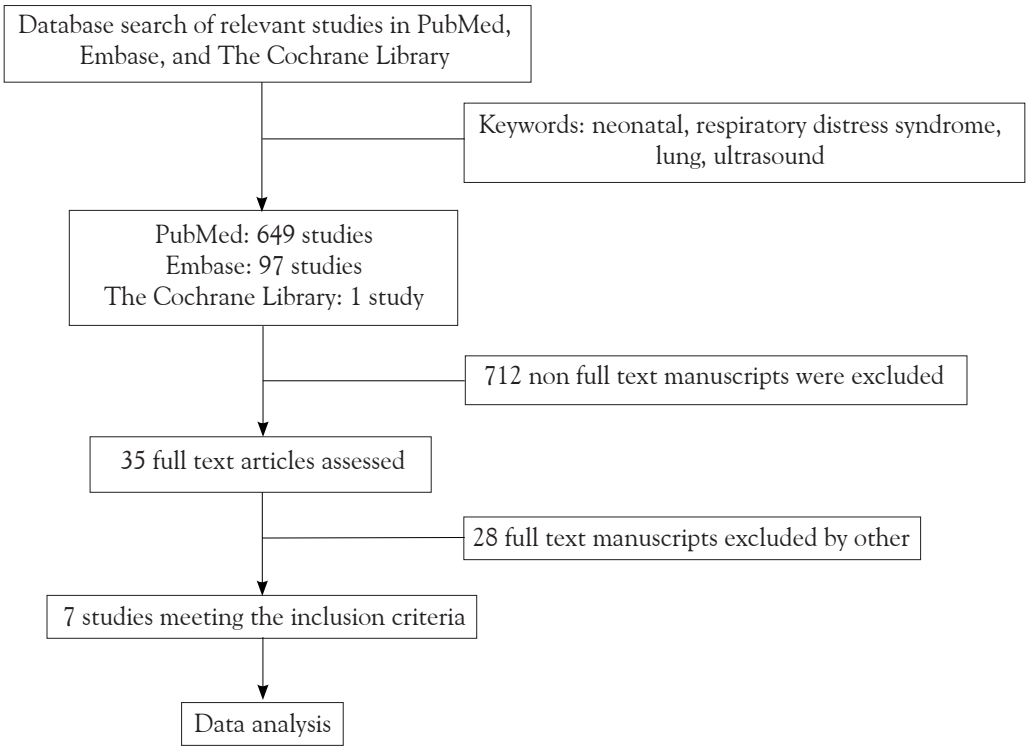

Figure 1. Flow chart study inclusion

Table 1. Primary data extracted from meta-analysis studies

Study	Year	Origin	Study type	Sample size	Gestational age, weeks	Male:female ratio	LUS	
							Sensitivity (%)	Specificity (%)
Bober *et al.*[19]	2006	Poland	Prospective	131	24-42	86/45	100	73
Copetti *et al.*[22]	2008	Italy	Case control	55	23-34	Unknown	100	100
Ahuja *et al.*[18]	2012	India	Prospective	88	25-32	50/38	84.2	88
Lovrenski J[21]	2012	Serbia	Prospective	47	23-36	Unknown	95	100
Liu *et al.*[23]	2014	China	Case control	100	27-41	62/38	100	100
Vergine *et al.*[25]	2014	Italy	Prospective	59	24-35	35/23	95.6	94
El-Malah *et al.*[20]	2015	Egypt	Prospective	100	36-42	66/44	98	92

Table 2. General characteristics of the studies

Study	Diagnostic methods	LUS operator	LUS technique	LUS equipment	LUS diagnostic criteria
Ahuja *et al.*[18]	Gastric aspirate test + clinical diagnosis + CXR	Radiologist	Transabdominal	HDI 3500 [advanced technologies laboratories (ATL) ultrasound, Bothell, WA, USA] (5-12MHz) curvalinier probe	Diffuse retrodiaphragmatic hyperechogenicity completely replacing the normal diaphragm
Bober *et al.*[19]	CRIB score + CXR + blood results	Physician	Transabdominal	Siemens SI 450, equipped with a sector 5MHz transducer	Retrophrenic hyperechogenicity with B-lines diverging radially
Copetti *et al.*[22]	Clinical diagnosis + CXR	Pediatrician + cardiologist	Transthoracic	Megas CVX Esaote, Medical system, Florence, Italy (10MHz linear probe)	Bi-lateral white lung, absence of spared areas, thickened and irregular pleural line
Liu *et al.*[23]	Clinical diagnosis + CXR + blood results	Expert physician	Transthoracic	High resolution line probe (11-12MHz) (GE voluson I or E6, USA)	Consolidation, pleural line abnormalities and bilateral white lung
Lovrenski[21]	Clinical diagnosis + CXR + blood results	Pediatric radiologist	Transthoracic + transabdominal	7.5MHz linear probe (Sonoline Adara, Siemens, Erlangen, Germany)	Consolidation; air bronchogram and B-lines
Vergine *et al.*[25]	Clinical diagnosis + CXR	Neonatologist	Transthoracic	Vivid-I Ge Medical Systems, Milan, Italy using a high res 10-12 MHz linear probe	Bi-lateral white lung, coalescent B-lines and thickened and irregular pleural line
El-Malah, *et al.*[20]	Clinical diagnosis + CXR	Radiologist	Transthoracic + transabdominal	Sonoline, Adara, Siemens, Erlangen, Germany using a 7.5MHz linear probe and 5MHz convex probe	B-lines, complete disappearance of white lung

CXR=chest X-ray

The sensitivity of lung ultrasound in diagnosing NRDS was figured in forest plot (**Figure 2**). The diamond sign is not across vertical lines (1.0) which means those studies has significant results. The I^2 of sensitivity obtained 86.5% then we took random effects models. It resolved heterogeneity in meta-analysis. **Table 3** explained the sensitivity proportion and the 95% CI for I^2 of each study. The proportion of Ahuja *et al.*,[18] Bober *et al.*,[19] El-Malah *et al.*,[20] Lovrenski,[21] Copetti *et al.*,[22] Liu *et al.*,[23] and Vergine *et al.*,[24] and were 84.2%, 100%, 95.6%, 100%, 100%, 98%, 95%, respectively. Then, it can be concluded that the total proportion of sensitivity revealed 97.2%.

The specificity of lung ultrasound in diagnosing NRDS was figured in forest plot (**Figure 3**). The diamond sign is not across vertical lines (1.0) which

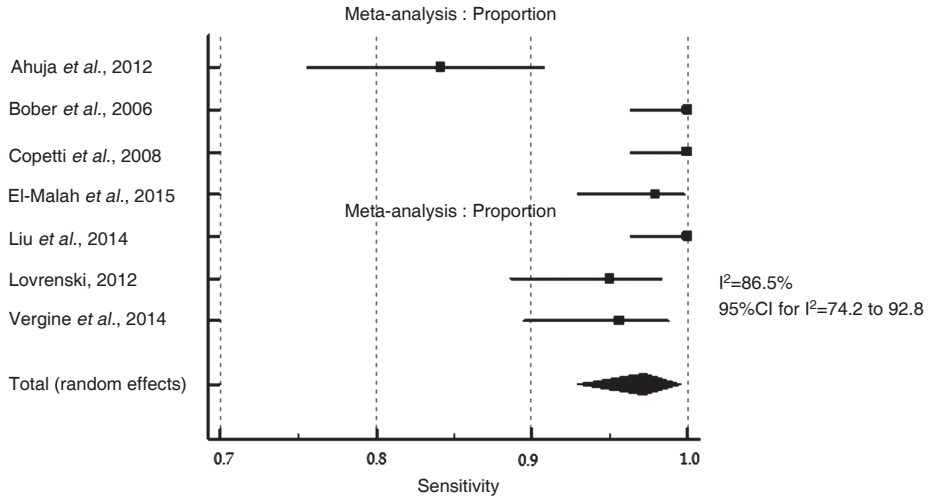

Figure 2. Forest plot: the sensitivity of lung ultrasound in diagnosis of NRDS

Table 3. Proportional meta-analysis: the sensitivity of lung ultrasound in diagnosis of NRDS

Study	Sample size	Proportion, %	95%CI	Weight, % [random effects]
Ahuja *et al.*[18]	100	84.2	75.5 to 90.7	14.3
Bober *et al.*[19]	100	100	96.3 to 100	14.3
Copetti *et al.*[22]	100	100	96.3 to 100	14.3
Liu *et al.*[23]	100	98	92.9 to 99.8	14.3
Lovrenski[21]	100	100	96.3 to 100	14.3
Vergine *et al.*[25]	100	95	88.7 tp 98.4	14.3
El-Malah, *et al*.[20]	100	95.6	89.5 to 98.7	14.3
Total (random effects)	700	97.2	92.9 to 99.5	100

Notes: Q=44.3, DF=6, significance level P<0.0001, I^2 (inconsistency)=86.5%, 95%CI for I^2=74.2 to 92.8

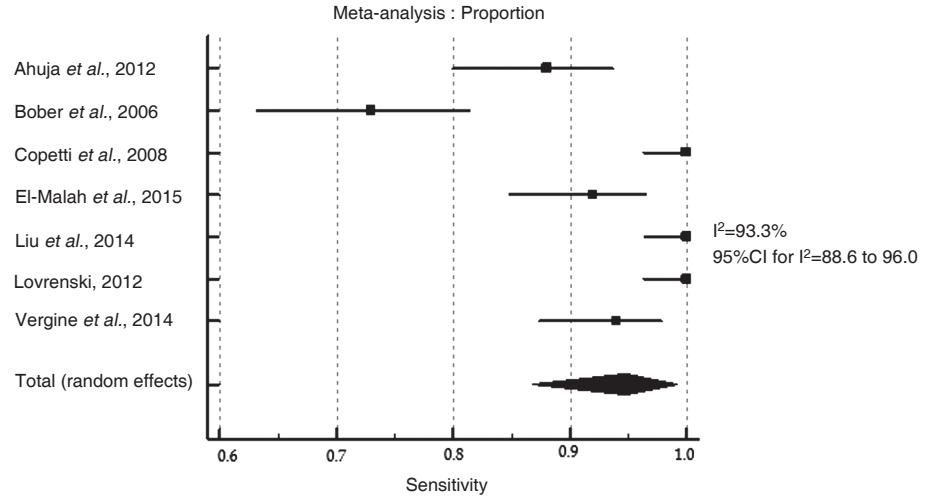

Figure 3. Forest plot: the specificity of lung ultrasound in diagnosis of NRDS

means those studies has significant results. The I^2 of sensitivity obtained 93.3% then we took random effects models. It resolved heterogeneity in meta-analysis. **Table 4** explained the specificity proportion and the 95% CI for I^2 of each study. The proportion of Ahuja *et al.*,[18] Bober *et al.*,[19] El-Malah *et al.*,[20] Lovrenski,[21] Copetti *et al.*,[22] Liu *et al.*,[23] and Vergine *et al.*,[24] and were 88%, 73%, 94%, 100%, 100%, 92%, 100%, respectively. Then, it can be concluded that the total proportion of specivicity revealed 94.8%.

The I^2 test results were greater than 50% across both forest plots (**Figure 2** and **Figure 3**). It confirmed a degree of heterogeneity among studies. Each subgroup was analyzed by Quadas-2 tool. The quality of the studies had an overall score of 30 out of 42 as shown in **Figure 4**.

Discussion

Neonatal respiratory distress syndrome is a common reason for NICU admission. It is the main cause of morbidity in preterm newborns with gestational age <37 weeks.[25] The standard diagnostic tools for diagnosing NRDS is a combination of clinical signs and symptoms as well as chest x-ray.[25,26] However, neonates may be vulnerable to excessive radiation of x-ray.[26] The radiation side effects lead to cataract

Table 4. Proportional meta-analysis: the specificity of lung ultrasound in diagnosis of NRDS

Study	Sample size	Proportion, %	95%CI	Weight, % [random effects]
Ahuja *et al.*[18]	100	88	79.9 to 93.6	14.3
Bober *et al.*[19]	100	73	63.2 to 81.4	14.3
Copetti *et al.*[22]	100	100	96.3 to 100	14.3
Liu *et al.*[23]	100	92	84.8 to 96.4	14.3
Lovrenski[21]	100	100	96.3 to 100	14.3
Vergine *et al.*[25]	100	100	96.3 to 100	14.3
El-Malah, *et al.*[20]	100	94	87.4 to 97.8	14.3
Total (random effects)	700	94.8	86.8 to 99.2	100

Notes: Q=89.2, DF=6, significance level P<0.0001, I^2 (inconsistency)=93.3%, 95%CI for I^2=88.6 to 96.0

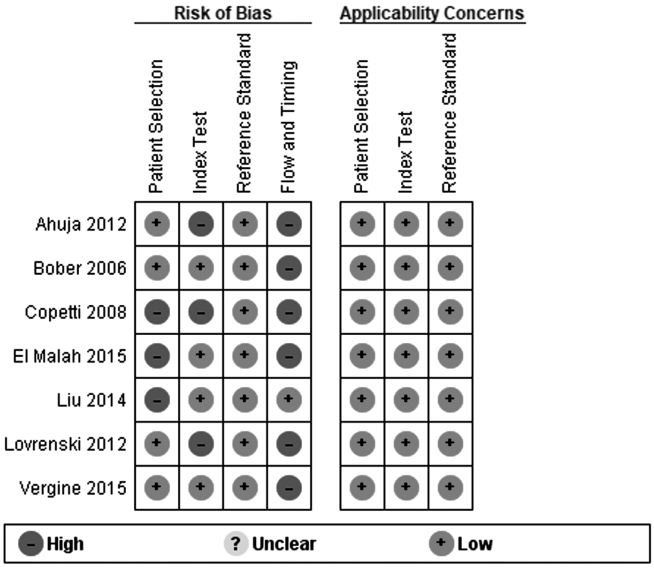

Figure 4. QUADAS-2: risk of bias and applicability concerns

and skin injury, as well as higher risk of hematology malignancy and cancer.[26,27] Infants of younger ages are more sensitive to radiation exposure.[27] Some studies using electrostatic discharge (ESD) showed that radiation exposure below 70 microGy can be tolerated by neonates.[27,28]

Chest x-ray (CXR) examination of NRDS reveals a ground glass appearance and air bronchogram.[29] Some experts say decreased pulmonary lucency as well as widespread net and grain high density shadows were not pathognomonic.[30,31] Hence, respiratory disorders in newborns could be diagnosed with others examinations.[32]

A previous study reported that CXR had sensitivity and specificity (35% and 82%, respectively), high sensitivity and specificity for diaphragmatic hernia and pneumothorax (100%). However it had 0% sensitivity and 98% specificity for congenital heart diseases and 0% sensitivity and 100% specificity for transient tachypnea.[33]

Lung ultrasound is an imaging examination with high accuracy, low cost, and no radiation side effects. Thus, some experts have suggested to use it as a diagnostic tool. It can be done on bedside and does not require sedation.[30,31] Lung ultrasound examination is considered to be superior by experts because it uses a transducer to emit radiofrequency waves, which reflect back to the transducer when encountering organ tissue. When air inside the alveoli is decreased as in NRDS, the transducer would receives a typical image which helps in diagnostic determination.

The seven studies included 580 neonates who may have had not only NRDS, but also any differential diagnoses with clinical signs similar to respiratory distress syndrome. The differences of study design could also have influenced subject inclusion. More than half of the sonographers in the seven studies were not blinded to clinical signs and chest x-ray examinations. This knowledge and the sonographers' skills could have influenced the final interpretation for diagnosis of NRDS, which could bias the diagnostic accuracy of the LUS.

The seven studies used clinical signs and symptoms and CXR to diagnose NRDS. Additional gastric aspiration test, clinical risk index for babies (CRIB) score, and blood gas analysis were used in four studies. These differences in clinical tests could also have led to bias in diagnostic accuracy of LUS. The NRDS reference standard still used clinical signs and symptoms as well as CXR in all studies.

The time duration between CXR and lung ultrasound varied among the studies, which could have biased the results due to the progressive severity of the disease. In addition, therapy or medication during the test could also lead to bias.

Our meta-analysis revealed >94% specificity and >97% sensitivity of lung ultrasonography as a diagnostic tool for NRDS. These high sensitivity and specificity values were closest to studies by Copetti *et al.* and Lovrenski that was omitted 100% numbers, [22,23] and in accordance with the most recent study by Al Kayat *et al.*[29] who reported 100% sensitivity and also found an 81% specificity of LUS for NRDS.

In conclusion, in diagnosing NRDS, lung ultrasound is superior to chest x ray as it has high sensitivity and specificity compared to those of chest x ray. Therefore, lung ultrasound can be considered as an alternative diagnostic tool for NRDS. Moreover, it is inexpesive, safe, and free radiation side effects.

Conflict of interest

None declared.

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Case Report

Laurence-Moon-Bardet-Biedl syndrome: a case report

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Laurence-Moon-Bardet-Beidl syndrome is a rare ciliopathic and pleiotropic human autosomal recessive genetic disorder.[1] In 1886, Laurence and Moon explained a case of a 7-year-old female with rod-cone dystrophy, hypogenitalism, mental retardation, obesity, and polydactyly. In 1920, Bardet described a 4-year-old female patient presented with rod-cone dystrophy, obesity, polydactyly (11 toes), and mental retardation.[1] Two years after Bardet's report, Biedl highlighted the complete scenario of clinical signs which includes skull abnormalities, anal atresia, mental deficiency, and gastrointestinal conflicts.[1] Since these discoveries, symptoms such as obesity, hypogonadism, retinal pigment defects, psychological hindrance, and polydactylismin in several conditions as combinations, frequently in children with normal parents (cousin marriages) has been termed as Laurence-Moon-Bardet-Biedl syndrome (LMBBS).[1]

Laurence-Moon-Bardet-Beidl syndrome (LMBBS) is a disorder with phenotypic and genetic heterogeneity.[2] The main features are obesity, polydactyly, pigmentary retinopathy, learning disabilities, various degrees of intellectual impairment, hypogonadism (in male) and renal abnormalities. Other clinical features include speech disorder, brachydactyly, developmental delay, polyuria and polydipsia, ataxia, poor coordination/clumsiness, diabetes mellitus, left ventricular hypertrophy, hepatic fibrosis and renal hypoplasia/dysplasia.[2] The most common feature is retinal dystrophy. The retinal appearance is quite variable, with typical retinitis pigmentosa being present in only a minority of cases.[1] Diagnosis of the condition is important for visual prognosis and low vision management.[2] The patients generally have onset of symptoms within the first 10 years of life and among them the first complaint is usually poor night vision.[1] Recent reports suggest that functional and morphological abnormalities are present in up to 90% of affected patients. The renal abnormalities occur with a spectrum of activity, often causing significant morbidity and autopsy data reveals it to be the major cause of mortality.[1] The management of Laurence-Moon-Bardet-Biedl syndrome involves a multidisciplinary approach and remains a challenge for clinicians. Here, we report a case of a 7-year-old girl presented with obesity, polydactyly, retinitis pigmentosa, and mental retardation. **[Paediatr Indones. 2019;59:349-52; doi: http://dx.doi.org/10.14238/pi59.6.2019.349-52]**.

Keywords: *obesity; retinitis pigmentosa; polydactyly; mental retardation*

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The Case

A 7-year-old girl, the firstborn of consanguineous parents, came to the pediatric outpatient department of 250 Bedded Hospital, Moulvibazar, Bangladesh, with complaints of gradual blurring of vision for the past two years and breathing difficulties during sleep for the past three years. The mother noticed that the child could not see well which was evident by repeated falls while walking on uneven surfaces and climbing stairs, groping for objects and the inability to see letters of the alphabet in books. No medical attention was sought for the vision problems because her action has been considered to be 'attention-seeking behavior' by her family members. She also suffered from breathing difficulty during sleep, blocked nasal passage, night cough, snoring, mouth breathing and recurrent sleep disturbance. No significant improvement was noted following repeated visits to local doctors. Hence, she visited the pediatric outpatient department of 250 Bedded Hospital for further evaluation and management. Her past medical history was uneventful, except that she had undergone surgical excision of an extra digit on her left hand due to infection at six months of age. Her birth, antenatal, natal and post-natal histories were normal. She was immunized as per the expanded program of immunization (EPI) schedule She had delayed developmental milestones as far back as her mother could remember, such as sitting at 8 months and walking at 2 years of age. Her mother had a history of one abortion at fetal gestation of four months. Her other sibling was healthy.

On general examination, the child had a puffy face, dull look and lack of attention to the surroundings. Vital signs were within normal limits. A skin survey revealed acanthosis nigricans over the neck, throat, axilla and groin. She had polydactyly (6 toes) on both feet. Her weight was 38 kg (weight-for-age >97th percentile), height was 121 cm (height-for-age 50th percentile) and body mass index (BMI) was 26 kg/m^2 (>95th percentile). Opthalmoscopic examination revealed retinitis pigmentosa. She was found to be mentally retarded on neurological examination. Other systemic examinations including genital examination revealed no abnormality.

Laboratory investigations including complete blood count (CBC), random blood sugar (RBS), alanine transaminase (ALT/SGPT), serum creatinin, lipid profile, free thyroxine (FT4), thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), luteinizing hormone (LH), urine routine and microscopic examination (RME) revealed normal findings. No abnormalities were seen on chest x-ray, electrocardiogram (EKG) or echocardiogram. Hepatomegaly with fatty changes in liver was detected

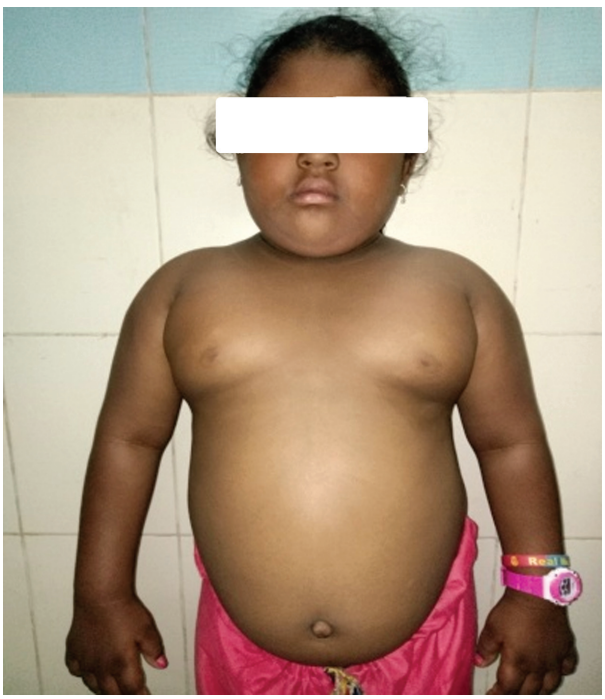

Figure 1. A 7 year-old case patient

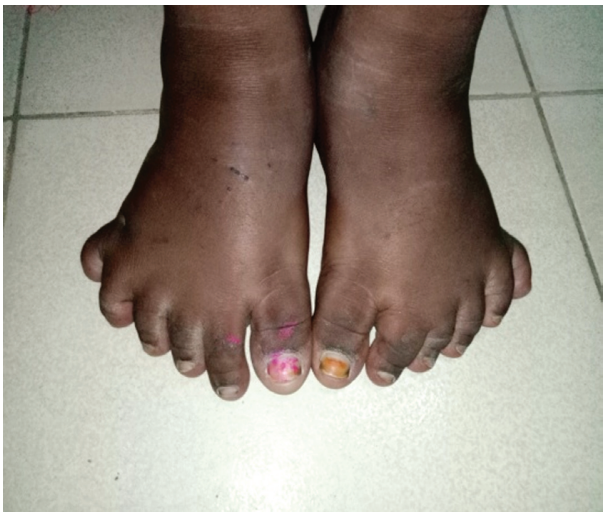

Figure 2. Polydactyly

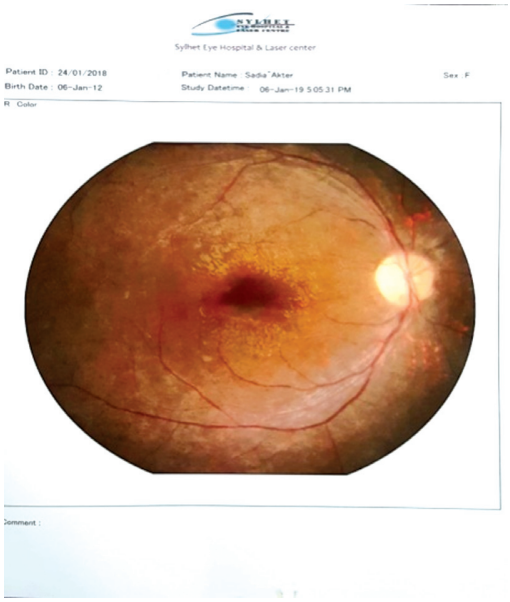

Figure 3. Retinitis pigmentosa (right eye)

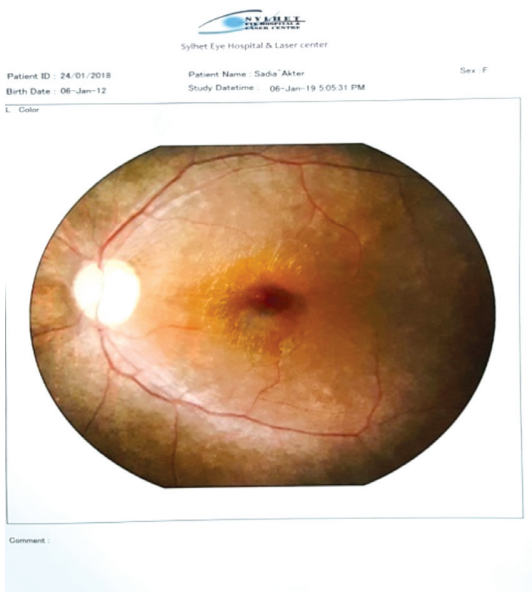

Figure 4. Retinitis pigmentosa (left eye)

by ultrasound. A nasopharynx lateral view x-ray revealed enlarged adenoids (grade-III). We diagnosed this patient to have Laurence-Moon-Bardet-Biedl syndrome on clinical grounds.

The patient was managed by a multidisciplinary approach and was regularly followed up. Proper counseling was done regarding the prognosis of the disease and the child's vision.

Discussion

Laurence-Moon-Bardet-Biedl syndrome (LMBBS) varies in its manifestation in different patients.[3] Apart from the primary features of obesity, post-axial polydactyly, renal abnormalities, mental retardation, pigmentary retinopathy, and hypogenitalism, other secondary manifestations exist.[3] These secondary characteristics include cardiac anomalies, neurological problems, nephrogenic diabetes insipidus, diabetes mellitus, dental anomalies, hypertension, speech disorders, behavioral problems, brachydactyly/syndactyly/clinodactyly, anosmia, lipid disorders, hepatic abnormalities and skin disorders.[3] In 1999, modified diagnostic criteria were defined in a British study of 109 LMBBS patients.[4] Patients who had at least 4 primary characteristics, or 3 primary and 2 secondary criteria, were identified as having LMBBS.[4] Our patient had four primary features, suggesting the diagnosis of LMBBS.

Bardet-Biedl syndrome (BBS) is now the standard term that replaced the older LMBBS, after it was found that the phenotypes overlap and may be allelic.[3] The prevalence of BBS was 1:160,000 in Europe and North America,[5] although higher incidences have been reported in isolated populations of Newfoundland (1:13,000)6 and Kuwait (1:17,000).[6] Diagnosis is usually not made early because the disease phenotypes are variable and slow-evolving.[3] Initial loss of the rod photoreceptors is followed by early macular involvement, with the degeneration of the cone cells, causing a gradual functional loss of vision.[3] Visual impairment, and probably poor school performance, are the common reasons for affected patients to seek treatment.[3] The visual pathology is an indicator pointing to the diagnosis in a child with other components of the syndrome. Our patient presented with gradual blurring of her vision.

Polydactyly and obesity are well-documented features of BBS.[3] Polydactyly, when present in BBS, is seen at birth. The incidence of obesity was reported to be 72 - 86% in the BBS population. Although the majority of patients have normal weight at birth, obesity usually sets in by infancy. Polydactyly in a child,

especially one with obesity, should stimulate a high index of suspicion for syndromic disorders.[3]

Mental retardation is a more disputed feature of BBS. Recently, objective IQ tests determined that only a minority of patients are mentally retarded. An IQ of 79 or below is found in 44% of BBS patients. The decrease in IQ level correlates with the presence of visual handicaps.[4] This finding was similar to our case.

Hypogenitalism is reportedly more frequent in BBS males than females.[4] In BBS females, genital abnormalities encompass a wide range, including hypoplastic fallopian tubes, uterus, ovaries, partial or complete vaginal atresia, absent vaginal orifice, or absent urethral orifice.[4] Renal failure is the major cause of morbidity and early mortality in BBS patients. A wide range of renal abnormalities has been described (chronic renal failure, parenchymal cysts, calyceal clubbing, fetal lobulation, scarring, unilateral agenesis, dysplastic kidneys, renal calculi, and vesicoureteric reflux).[4] These features were absent in our reported case.

Bardet-Biedl syndrome (BBS) is genetically heterogeneous, with 12 BBS genes (BBS1-12) identified to date.[7] As these BBS proteins are components of the centrosome that influences ciliary transport, this syndrome is categorized under the spectrum of ciliopathies.[8] Genetic analysis was not carried out in our case.

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