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Original Article

Triiodothyronine levels and mortality in children with sepsis and septic shock

Cynthea Prima Destariani, Munar Lubis, Melda Deliana, Gema Nazri Yanni

Abstract

Background Sepsis is the most common cause of death in infants and children. It can cause hormonal imbalances, such as euthyroid sick syndrome (ESS), which may increase the risk of death.

Objective To evaluate a possible correlation between the level of triiodothyronine (T3) and mortality in children with sepsis and septic shock.

Methods An observational cohort study was conducted on 80 children with sepsis and septic shock from October 2015 until January 2016 in Haji Adam Malik General Hospital, Medan, North Sumatera. Subjects underwent PELOD score and T3 examination on the first day admitted in Haji Adam Malik General Hospital. Chi-square test was used to analyze for a correlation between the T3 values and mortality.

Results Of the 80 consecutive subjects, 39 (48.75%) had low T3 level on the first day. Of these 39 children, 36 (92.3%) died. Subjects with low T3 level had a 6.31 times higher risk of mortality(PR 6.31; 95%CI 2.99 to 13.28; P<0.001). Of the 31 subjects with high PELOD score, 23 (74.2%) had decreased T3 (PR=2.27; 95%CI 1.45 to 3.57; P<0.001).

Conclusion Low T3 levels have significant relationship with mortality in children with sepsis and septic shock. **[Paediatr Indones. 2018;58:20-4 ; doi: http://dx.doi.org/10.14238/pi58.1.2018.20-4]**.

Keywords: *triiodothyronine; mortality; sepsis; septic shock; children*

Sepsis is the body's response to various systems of systemic inflammatory response syndrome (SIRS) with evidence of infection or suspected infection.[1-3] Sepsis is a common cause of death in infants and children. The incidence of severe sepsis and septic shock has risen in the past 30 to 40 years.[4] *The World Health Organization* (WHO) reported that 70% of the 8 million deaths of children under 5 years in developing countries was due to infectious diseases that mostly ended with sepsis. The incidence of sepsis worldwide reached 0.56 per 1,000 children and 5.6 per 1,000 babies with the presentation of deaths by 10.6 %.[5] *The 2007 Basic Health Research Report* showed that 20.5% of infant deaths was caused by sepsis.[6] In 2009, the Division of Pediatric Emergency, Department of Child Health, Cipto Mangunkusumo Hospital (RSCM), Jakarta, reported a sepsis incidence of 19.3% of 502 pediatric

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patients admitted to the pediatric intensive care unit (PICU), with a mortality rate of 10%.[7] Mortality due to neonatal sepsis in Haji Adam Malik General Hospital, Medan, from 2008 to 2010 was 32.9%.[8]

Hemodynamic disturbances occur in sepsis, such as cardiovascular and hormonal balance disorders. Hormonal changes that frequently occur in sepsis are often from the thyroid, in the form of euthyroid sick syndrome (ESS) or non-thyroidal illness syndrome (NTIS).[9] These syndromes are characterized by decreased levels of thyroid hormones, but without the impaired function of the thyroid hormone that occurs in non-thyroidal severe systemic disease. Changes in thyroid hormone eventually lead to impaired oxygen consumption and hematopoesis, as well as cardiovascular, sympathetic nervous, respiratory, and digestive system problems, which ultimately lead to organ system failure and death.[10] A Jakarta study in 2012 showed that thyroid hormone levels were low in a substantial group of patients before surgery. This abnormal thyroid hormone levels in this study group can be defined as euthyroid sick syndrome (ESS).[11]

Critical illness is characterized by the existence of complex and multiple changes in thyroid pathways. As the patient condition worsens, not only does triiodothyronine (T3) decrease, but thyroxine (T4) and thyroid stimulating hormone (TSH) also do. Decreased levels of T4 and TSH are an indication of worsening disease and poor prognosis, about 80% of subjects, especially in patients with T4 <3μg/dL. Decreased levels of thyroid hormones are still a matter of controversy to this day. Studies in Greece and the United States reported that decreased T4 and TSH levels affect mortality in sepsis and septic shock.[12,13] Study in the Netherlands reported that the decrease in T4 levels affects mortality, but studies in Belgium found that decreased levels of T3 affected mortality. Decreased T3 causes changes in thyroid hormone metabolism.[13-16] This study was aimed to evaluate a possible correlation between the level of triiodothyronine (T3) and mortality in children with sepsis and septic shock.

Methods

This cohort study was conducted in Haji Adam Malik General Hospital, Medan, North Sumatera, from October 2015 until January 2016. Eighty children with sepsis and septic shock aged 1 month to 18 years were evaluated. According to *Surviving Sepsis Campaigne*, sepsis was a systemic inflammatory response syndrome (SIRS) caused by infection, both proven by blood cultures and suspected clinical infection and septic shock was a condition due to severe sepsis resulting in disruption of several organs in the body accompanied by circulatory disorders. Normal level of T3 was 1.4 to 4 nmol/L, and PELOD score with high mortality was > 20 and low mortality was < 20.

Subjects were collected by consecutive sampling. The study was done by conducting interviews the parents to obtain the history of preceding illness. We assessed the degree of disease severity by *Pediatric Logistic Organ Dysfunction* (PELOD) score. Subjects underwent weight, body length, and laboratory parameter measurements (complete blood count/CBC, thyroid hormone, qualitative C-reactive protein/CRP, procalcitonin, and blood cultures). Examination of thyroid hormones on the first day and the fourth day admitted in Haji Adam Malik Hospital Medan was conducted. Patient monitoring was done within 7 days. Exclusion criteria were patients with hypothyroid and hyperthyroid disease. This study was approved by the Medical Ethics Committee of the University of Sumatra Utara Medical School.

The collected data were processed, analyzed, and presented by *SPSS software*. The significance level was P<0.05. Bivariate analysis (Chi-square test) was performed to assess for a correlation between thyroid hormone values and death. The correlation between thyroid hormone level and PELOD was analyzed by Mann-Whitney test.

Results

Eighty patients with sepsis (40 subjects) and septic shock (40 subjects) were included (**Table 1**). Of the 39 subjects that showed decreased T3, 36 (93.2%) did not survive (PR=6.31; 95%CI 2.99 to 13.28; P<0.001). The prevalence ration (PR) indicated that subjects with decreased T3 level had a 6.31 times higher chance of mortality (**Table 2**). Of the 31 subjects with high PELOD score, 23 (74.2%) had decreased T3 (PR=2.27; 95%CI 1.45 to 3.57; P<0.001). Statistical analysis indicated that subjects

with high PELOD scores had a 2.27 times higher chance of low T3 level.

Table 1. Demographic characteristics of subjects

Variables	Septic shock (n=40)	Sepsis (n=40)
Sex, n(%)		
Male	23 (57.5)	23 (57.5)
Female	17 (42.5)	17 (42.5)
Mean age (SD), years	8.15 (5.81)	7.6 (5.33)
Length of stay, n(%)		
< 7 days	19 (47.5)	18 (45)
> 7 days	21 (52.2)	22 (55)

Table 2. Relationship between decreased T3 level with mortality

PELOD score, n(%)	Decreased T3		PR (95%CI)	P value
	Yes (n=39)	No (n =41)		
High	23	8 (25.8)	2.27	< 0.001[a]
Low	16	33 (67.3)	(1.45 to 3.57)	

[a]Chi-square test

Table 3. Relationship between PELOD score and decrease in T3 level

PELOD score, n(%)	Decreased T3		PR (95%CI)	P value
	Yes (n=39)	No (n =41)		
High	23	8 (25.8)	2.27	< 0.001[a]
Low	16	33 (67.3)	(1.45 to 3.57)	

[a]Chi-square test

Discussion

At the beginning, a decrease in T3 occurs due to a defect of enzyme 5'-deiodinase that converts T4 to T3, a decrease in the number of thyroid receptors that are mediated by interleukin 1β and their thyroid binding protein inhibitor, as well as an increase in TNF-α during the critical illness.[17] This T3 decrease early in infection (36-72 hours) is followed by a TSH increase due to peripheral thyroid hormone. This feedback mechanism is called the restoration of metabolic activity, and it is responsible for returning T3 levels to normal.[18] These T3 fluctuations are consistent with a study in the Netherlands which found that 44 children in PICU with low T3.[12] Similarly, we found decreases in T3 and T4 levels, followed by an increase in TSH thyroid axis, called euthyroid sick syndrome.

A Turkish study in 2004 showed a decrease in T3 and T4 in sepsis patients.[19] Another study in Jakarta in 2014 showed that thyroid hormone declined in patients with sepsis, especially T3 values.[20] In our study, 80 children with sepsis and septic shock had their T3 levels measured on the first day and the fourth day of hospitalization. We found decreased T3 in the sepsis and septic shock groups at both days.

In adult patients with serious illness, euthyroid sick syndrome can be used to predict the severity of disease; however, in the pediatric population, there is a lack of data on changes in thyroid hormones, as a predictor of disease severity. Some studies have shown that major changes in thyroid hormone levels indicate severity of illness and rates of survival.[24]

Changes in thyroid hormone will always appear in patients with serious illness as a favorable adaptation response and without intervention. In the acute phase (within 24 hours after infection), enzyme dysfunction D2 and D3 which causes the increased in activity of T3 is not formed; in this condition, more T4 is converted to inactive T3. In a chronic or severe acute condition, central dysfunction of the hypothalamus occurs. This situation is temporary, generally lasting 36-72 hours, and normalizing again after 72 hours, along with recovery from illness.[25] In our study, we found that the decrease in T3 levels of patients with sepsis can occur within a few hours during the acute phase.

T3 hormone plays a role in DNA replication and binds to the D3 enzyme to forestall cell apoptosis. The reduction in T3 leads to increased levels of D3 free-enzyme and apotosis of cells. Poor outcomes may result from this aggravating of the disease condition.[26]

A Dutch study showed that low thyroid hormone in critically ill patients can cause death.[27] Also, a study in Turkey reported that the levels of T3, T4, FT3 and FT4 were lower in children with septic shock, compared to sepsis-related mortality of patients with sepsis.[18] In addition, a European study in 2011 showed that the decrease in thyroid hormones was related to patient prognosis in those with sepsis or septic shock, although not always consistent.[28] A Semarang study showed no significant difference in the levels of thyroid hormone in sepsis patients with mortality.[29] However, a 2014 Jakarta study showed that thyroid hormone

declines in septic patients, especially the value of T3. High PELOD value can be used as a prognosticator in PICU cases of sepsis, despite the lack of a relationship between PELOD and decreased thyroid hormone levels.20 In our study, there was significant correlation of T3 impairment with death. Of the 39 subjects with decreased T3 levels, 36 (92.3%) of them died (PR= 6.31; 95%CI 2.99 to 13.28; P<0.001), which indicated that children with decreased T3 level had a 6.31 times higher chance of mortality.

In patients with severe sepsis and septic shock, FT4 and TSH levels decline because the decrease in plasma thyroxine-binding globulin (TBG) eventually decreases thyroid binding plasma capacity.[13] In our study, all subjects sepsis levels were ignored and the ratio of rT3/T3 and the inotropic agents were not considered. Dopamine has a suppressive effect on pituitary TSH secretion, by directly inhibiting pituitary function through dopamine inhibitor receptors, which causes the decrease in TSH secretion.[30]

Prognostic accuracy of death in critically ill patients treated in the ICU has several advantages. *The Acute Physiology and Chronic Health Evaluation* (APACHE) II has generally been used as a method of prognostic outcome in ICU patients. This measurement does not assess the hormonal response to the underlying disease, especially cortisol and thyroid hormone concentration, which have been significantly associated with mortality in critically ill patients. Mortality in ICU patients is best predicted by a combination of T3 and TSH levels, and APACHE II scores determined at the time patients are sent to the ICU.31 However, we used PELOD score to predict mortality: 31 subjects had high PELOD scores and 23 of them (74.2%) had decreased T3 levels compared to 51.25% in those with low PELOD scores (PR=2.27; 95%CI 1.45 to 3.57; P<0.001). These results indicated that children with high PELOD scores had a 2.27 times higher chance decreased T3, compared to those who had low PELOD scores.

The limitations of our study were not distinguishing the degrees of sepsis, not counting the rT3/T3 ratio, and not taking into account the use of inotropes.

In conclusion, low T3 levels have significant relationship with mortality in children with sepsis and septic shock.

Conflict of Interest

None declared.

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Original Article

Nutritional therapy and caloric achievement within the first week of PICU admission

Melia Yunita, Desy Rusmawatiningtyas, Titis Widowati

Abstract

Background Nutritional therapy is an important aspect in managing PICU patients. Careful decisions should be made regarding initiation, route of administration, and achievement based on caloric requirements. Many conditions could affect the application of nutritional therapy.

Objective To investigate the implementation of nutritional therapy during the 1st week after PICU admission.

Methods We conducted a retrospective study involving 156 children aged 1 month-18 years who were hospitalized for at least 4 days in the PICU during the period of January 1, 2015 to December 31, 2015. Subjects were divided into three groups according to initiation time of caloric administration, which were: category I (within the first 24 hours of PICU admission), category II (within the first 25-48 hours of PICU admission), and category III: (more than 48 hours after PICU admission). Caloric requirement was calculated using the Caldwell or Schofield formula, whilst caloric achievement was figured up from PICU daily monitoring sheets containing nutritional therapy given to the subjects.

Results Of 131 subjects, 72 (55%) had good nutritional status and 59 (45%) children had malnutrition. Caloric administration was initiated within 24 hours of admission in 101 (77.1%) patients, of whom 90 (89.1%) patients received enteral feeding. Nineteen (14.5%) patients received their initial calories within 25-48 hours of admission, with 16 (84.2%) using the enteral route. At the 4th and 7th days of hospitalization, 93 (71%) and 107 (81.7%) patients achieved >70% of their caloric requirements. Delays in feeding initiation were due to shock, gastrointestinal bleeding, inotropic support, and feeding intolerance, which reduced caloric achievement.

Conclusion Most patients receive nutritional therapy in the first 48 hours after PICU admission and achieve >70% of their caloric requirements at the 4th day of hospitalization. The enteral route is preferred. Delayed initiation of nutritional therapy reduces caloric achievement. **[Paediatr Indones. 2018;58:13-9; doi: http://dx.doi.org/10.14238/pi58.1.2018.13-9]**.

Keywords: *nutritional therapy; malnutrition; PICU; caloric achievement*

Critically ill conditions have been associated with catabolic stress that leads to increased morbidity and mortality if not managed properly. Nutritional support is an important aspect in management of patients in the PICU. Adequate nutritional therapy is needed to maintain the integrity of intestinal mucosa, improve the structure and function of the gastrointestinal system, hasten wound healing, decrease catabolic response to trauma, as well as reduce infection and early mortality.[1,2] The route selected for caloric administration, either oral, enteral, parenteral, or a combination, depends on the condition of the patient's gastrointestinal tract and its ability to absorb nutrients. Each of these routes has its own indications, benefits, durations of administration, and complications that may occur. The gastrointestinal tract in critically ill children often does not function optimally, so either the enteral or parenteral route is preferred. Many studies reported that enteral nutrition provides better outcomes than parenteral nutrition.[2–4]

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Enteral nutrition within the first 48 hours in adult patients with a mechanical ventilator was associated with a decrease in the mortality rate of up to 20%.3 Another study reported that adults with septic shock and mechanical ventilator who received enteral nutrition within the first 48 hours showed shorter durations of hospitalization and ventilator usage.[5]

The caloric achievement does not always meet the planned caloric requirement. Mehta et al. found that the achievement of caloric needs in PICU patients was only 51% of their caloric requirement on the 6th day of hospitalization.[4] Another study reported that on the 9th day of hospitalization, only 50% of patients reached their caloric needs.[6] These studies illustrate the constraints of achieving adequate nutrition children admitted to the PICU. Reasons that may be associated with these conditions were fluid restriction, enteral and parenteral route disruptions, feeding intolerance, and interruptions due to diagnostic procedures that require fasting, such as radiological examinations. Failure to achieve caloric need has been associated with longer length of stay and higher mortality rate.[4,7]

We aimed to describe the implementation of nutritional therapy in PICU patients including initiation time, route of administration, caloric achievement, as well as conditions that affected therapy. We found that the average length of stay in the PICU, Dr. Sardjito General Hospital in 2015 was 7 days, so we recorded nutritional therapy during this period of time.

Methods

We conducted a retrospective study in children aged 1 month to 18 years who were hospitalized in the PICU at Dr. Sardjito General Hospital, Yogyakarta, for at least 4 days from January 1, 2015 to the December 31, 2015. We chose a duration of at least 4 days based on the physiological phase of metabolic stress in critically ill patients. The ebb phase starts immediately and is followed by a flow phase after 48-72 hours. In the second phase, substrate mobilization to produce energy occurs in order to prevent the effect of auto-cannibalism, so the human body needs enough nutrition to provide the substrate. Data were collected from patients' medical records using a questionnaire covering demographic data (age and sex) and clinical data which included date of admission to and discharge from the hospital, date of admission to and discharge from the PICU, weight and height at admission, categories of primary disease (non-surgical, digestive surgical, or non-digestive surgical), course of the disease during hospitalization (fluid restriction, feeding intolerance, shock, gastrointestinal bleeding, availability of central access, absence of an intravenous line, and investigations/medical procedures that required fasting), as well as the patient's condition when discharged from the PICU (survived or died). Nutritional therapy data were obtained from medical records and daily monitoring sheets from the PICU. Caloric requirement was calculated using the *Caldwell* formula for patients with mechanical ventilator and the *Schofield* formula for patients without mechanical ventilator.[8–10] Patients with incomplete medical records were excluded from the study.

Subjects were divided into three groups based on initiation time of caloric administration, which were category I: patients who started nutritional therapy within the first 24 hours of PICU admission, category II: patients who started nutritional therapy within the first 25-48 hours of PICU admission, and category III: patients who started nutritional therapy at more than 48 hours after PICU admission. Route of administration was classified into 2 groups according to the route selected to initiate caloric administration, either enteral or parenteral route. Meanwhile, we set a caloric achievement cut-off value of 70% of the caloric requirement. This cut-off was derived from a previous study that stated that energy intake >66.7% resulted in significantly lower mortality.[4]

The sample size was calculated using the simplified *Lameshow* binomial formula for survey study based on an estimation that 50%[6] of patients could achieve the caloric requirement, with precision of 10%. This calculation resulted in a minimum required sample size of 100.

Data were analyzed using a *SPSS version 20* software. Univariate analysis was conducted to describe the characteristics and distribution of research data. The variables were compiled in the form of descriptive narrative and tables. This study received approval from the Medical and Health Research Ethics Committee of the Universitas Gadjah Mada Medical School/Dr. Sardjito General Hospital.

Results

There were 156 children aged 1 month to 18 years who were treated in the PICU at Dr. Sardjito General Hospital for at least 4 days during the one-year study period. Twenty-five patients were excluded because of incomplete medical records, leaving a total of 131 subjects. Characteristics of the study subjects are shown in **Table 1**. Of our subjects, 55% had good nutritional status and 45% had malnutrition. In the group of patients who died, as many as 59.6% were malnourished, with undernourished as the highest proportion (32.7%).

Our subjects were distributed into the following categories of nutritional therapy initiation: 101 (77.1%) patients in category I, 19 (14.5%) patients in category II, and the remaining 11 (8.4%) patients in category III. The enteral route was used more frequently than the parenteral route in patients from category I [90 patients (89.1%) *vs.* 11 patients (10.9%), respectively] and category II [16 patients (84.2%) vs. 3 patients (15.8%), respectively]. Patients from category III differed, in that the parenteral route was used more frequently than the enteral route [7 patients (63.6%) vs. 4 patients (36.4%), respectively].

Thirty patients who received caloric administration after the first 24 hours (categories II and III) consisted of 28 (93.4%) non-surgical patients, 1 (3.3%) digestive surgical patient, and 1 (3.3%) non-digestive surgical patient. The 11 patients who started caloric administration after 48 hours (category III) of PICU admission were all non-surgical patients. The reasons for delays in feeding initiation in both groups are shown in **Figure 1**.

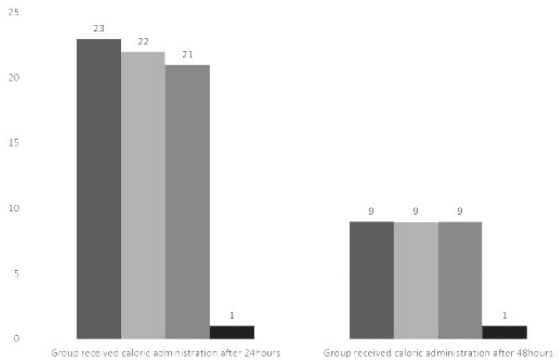

Figure 1. Reasons for withholding caloric administration

Table 1. Basic characteristics of study subjects

Characteristics	Total N=131	Category I n=101	Category II n=19	Category III n=11
Sex, n(%)		56 (55.4)	12 (63.2)	4 (36.4)
Male	72 (55)			
Median age (range), month	36 (1-197)	14 (1-197)	82 (11-182)	101 (360173)
Nutritional status, n(%)				
Good	72 (55)	54 (53.5)	12 (62.3)	6 (54.5)
Undernourished	26 (19.8)	24 (23.8)	1 (5.3)	1 (9.1)
Severe malnutrition	23 (17.6)	18 (17.8)	1 (5.3)	4 (36.4)
Overweight	4 (3)	1 (1)	3 (15.8)	-
Obese	6 (4.6)	4 (4)	2 (10.5)	-
Primary disease, n(%)				
Non surgical	113 (86.3)	85 (84.2)	17 (89.5)	11 (100)
Digestive surgical	10 (7.6)	9 (8.9)	1 (5.3)	-
Non-digestive surgical	8 (6.1)	7 (6.9)	1 (5.3)	-
Mechanical ventilator, n(%)				
No	47 (35.9)	33 (32.7)	9 (47.4)	5 (45.5)
Yes	84 (64.1)	68 (67.3)	10 (52.6)	6 (54.5)
Outcome, n(%)				
Survived	79 (60.3)	54 (53.5)	16 (84.2)	9 (81.8)

Classification of nutritional status was based on 2006 WHO Z-score weight per height criteria for children aged < 5 years old, body mass index (BMI) per age criteria for children > 5 years old.
Good nutritional status: weight per height or BMI per age Z-score of -2 SD < Z score < 2 SD;
Undernourished: weight per height or BMI per age Z-score of -2 SD < Z score < -3 SD;
Severe malnutrition: weight per height or BMI per age Z-score of < -3 SD;
Overweight: weight per height or BMI per age Z-score of 2 SD < Z score < 3 SD;
Obese: weight per height or BMI per age Z-score > 3 SD.

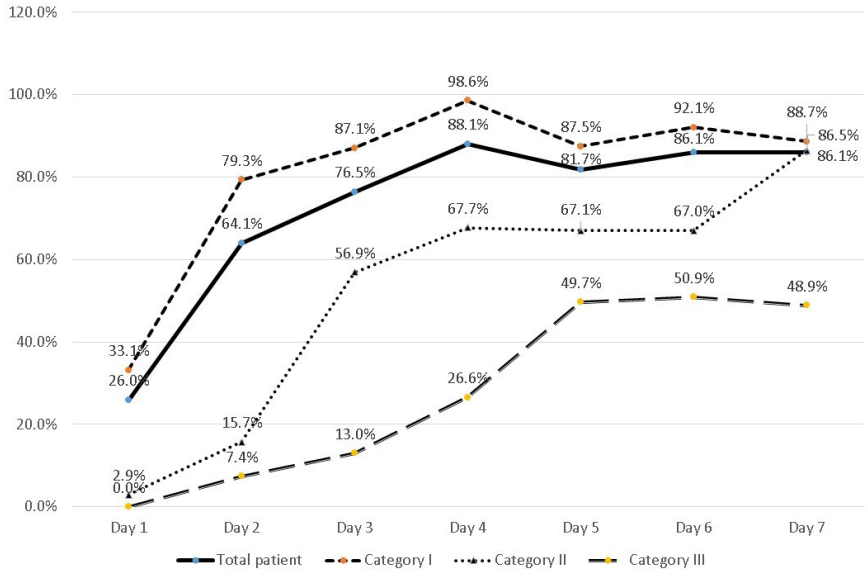

Figure 2. Daily mean percentage of caloric achievement
Total patients: days 1-4: 131 patients, day 5: 109 patients, day 6: 95 patients, day 7: 84 patients.
Category I: day 1-4: 101 patients, day 5: 86 patients, day 6: 76 patients, day 7: 69 patients.
Category II : day 1-4: 19 patients, day 5: 14 patients, day 6: 13 patients, day 7: 10 patients.
Category III: day 1-4: 11 patients, day 5: 9 patients, day 6: 6 patients, day 7: 5 patients.

On the 4th day of hospitalization, 82 (62.6%) patients achieved more than 70% of their caloric requirements through the enteral route, and 93 (71%) patients achieved such through enteral and/or parenteral routes. On the 7th day of hospitalization, 99 (75.6%) patients reached 70% of their caloric target through the enteral route and 107 (81.7%) patients through enteral and/or parenteral routes. The daily mean percentage of caloric achievement is shown in **Figure 2** and the proportion of patients who achieved more than 70% of the caloric requirement on the 7th day of hospitalization based on time of initial feeding is shown in **Figure 3**.

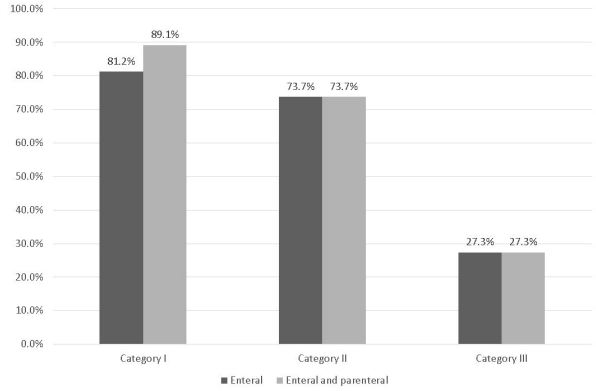

Figure 3. Proportion of patients who achieved >70% of caloric requirements on the 7th day of hospitalization, based on time of initial feeding

Discussion

The proportion of malnourished patients in our study was 45%, higher than in several previous studies which reported 15-30%.[7-9] This discrepancy could be due to our setting, since most previous studies were conducted in developed countries. Most children treated in the PICU become malnourished during hospitalization because of their hypercatabolic condition caused by sepsis, shock, and inflammation. This condition may rapidly decrease body mass, weaken the function of vital organs, and destroy the immune system, all of which absorb 30-50% of their energy.[8-10]

Enteral nutrition is the first choice in patients without gastrointestinal disorders because using the enteral route can prevent atrophy of intestinal mucosa and reduce intestinal permeability to prevent bacterial

translocation and sepsis.[11] A previous study noted that the administration of enteral nutrition in the first 12-24 hours of hospitalization may effectively increase cumulative energy, as well as reduce the incidence of infection and length of hospitalization.[7] Of the subjects receiving initial nutrition within 24 and 24-48 hours, 89.1% and 84.2% of patients received first caloric intake through the enteral route, respectively. Previous studies have provided evidence that early enteral nutrition is an effective way to prevent the occurrence of stress-related mucosal damage (SRMD), an effect of metabolic stress that occurs in critically ill children. Enteral nutrition may increase blood flow to the intestine, increase perfusion, increase gastric pH, and prevent ischemia that can cause SRMD and, consequently, trigger gastrointestinal bleeding.[11-13] Parenteral nutrition has a higher risk of becoming a source of infection than the enteral route. However, in patients who cannot tolerate the enteral route, the parenteral route should be the choice of administration.[12] In our study, the initial feeding by parenteral route was given in less than 16% of patients.

We found the common reasons for withholding caloric administration to be shock, gastrointestinal bleeding, and use of inotropes. This finding was consistent with a previous study which reported such conditions to be the reasons for delaying enteral nutrition, in order to reduce the risk of intestinal necrosis.[17] The latest consensus of the *American Society for Parenteral and Enteral Nutrition* (ASPEN) 2016 was in agreement, while the consensus for pediatric patients in 2009 had not mentioned about this yet.[18,19] However Panchal *et al.* did a retrospective multicenter study with children treated in the PICU for 4 days or more, who received at least one type of vasoactive drug. They showed that enteral nutrition was well tolerated in 78% of patients and there was no significant difference in gastrointestinal side effects between the group of patients who received enteral nutrition within 48 hours and the group of patients who did not. Nevertheless, the limitations of their study were the selective samples, differences in patients' basic characteristics, and differences in the vasoactive inotropic score (VIS) between the two patient groups.[20] Further prospective study will be needed to determine the effect of enteral nutrition in patients with hemodynamic instability and those who use of vasopressors. A Korean study reported 59 cases of delayed enteral nutrition due to gastrointestinal bleeding, which later revealed that only 7 (11.9%) of these cases had active gastrointestinal bleeding. Most of the cases (52 cases or 79.7%) were actually a blood clot.[21] A specific definition of gastrointestinal bleeding is needed because it affects patient management. The SRMD was a common cause of upper gastrointestinal bleeding within the first 24 hours of hospitalization in 75-100% of patients admitted to the PICU. Enteral nutrition was reported safe in patients with SRMD, and even reduced the likelihood of further bleeding; while in patients with massive upper gastrointestinal bleeding, enteral nutrition was contraindicated until the patient was stable and had low risk of recurrent bleeding.[14] The limitation of our study was its retrospective design. Diagnosis of gastrointestinal bleeding was solely based on the information available in the medical records with limited detail on severity.

We observed that delayed feeding initiation reduced caloric achievement, especially when started at more than 48 hours after admission. These results were consistent with those of Martinez *et al.* who found that one-day delay of initiating caloric intake decreased caloric achievement by 16.66%.[22]

In conclusion, during the first week of treatment, most patients in the PICU at Dr. Sardjito General Hospital, Yogyakarta receive caloric administration within the first 48 hours of hospitalization and achieve more than 70% of the caloric requirement on the 4th day of admission. The enteral route is the preferred route to initiate caloric intake. Delays in feeding initiation reduce the percentage of caloric achievement.

Conflict of Interest

None declared.

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Original Article

Early detection of cerebral palsy in high-risk infants: diagnostic value of primitive and developmental reflexes as well as ultrasound

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Abstract

Background The incidence of cerebral palsy (CP) has increased due to better survival of high-risk babies. A simple assessment method is needed for the early detection of CP, which can be performed by general practitioners and pediatricians in daily practice.

Objectives To assess motor delay, primitive and developmental reflexes, and cerebral ultrasound abnormalities as simple methods for early detection of CP in high-risk infants. We also aimed to evaluate the ease and consistency of the methods for use in daily practice, as well as determine risk factors associated with CP.

Methods A prospective cohort study was done on 150 high-risk babies starting from the age of 4 months up to 12 months. We obtained subjects' histories of motor ability and assessed primitive reflexes and postural reactions at the ages of 4, 6, 9 and 10 months. The diagnosis of CP was established at 6 and 12 months of age. We also determined Kappa test for inter-rater reliability between pediatric residents and the pediatric neurologist.

Results In 88.7% of subjects, CP was detected in the first 6 months. At 4 months, positive palmar reflex, head lag, and fisting were predictive of CP at 6 months of age. Motor delay, positive palmar grasp reflex, head lag, fisting, and absent protective extension reflex at 6 months were predictive of CP at 12 months. At 9 to 10 months, motor delays, absent protective extension reflex, and negative parachute reaction were predictive of CP at 12 months. Cerebral ultrasound abnormalities were predictive of CP at 6 and 12 months of age. Kappa test result was 0.9, indicating the ease and consistency of these methods for daily medical practice.

Conclusion Cerebral palsy can be detected as early as the first 6 months of life. Assessment for motor delays, physical examination for asssessing primitive and developmental reflexes, and cerebral ultrasound can be used for this purpose. **[Paediatr Indones. 2018;58:5-12 ; doi: http://dx.doi.org/10.14238/pi58.1.2018.5-12]**.

Keywords: *early detection; cerebral palsy; cerebral ultrasound; motor delay; postural reaction; primitive reflex*

The incidence of cerebral palsy (CP) is 1.2 to 2.5 per 1,000 live births. Several factors, including prematurity, influence the occurrence of CP.[1] In Canada, the mortality of premature infants has declined from 256 per 1,000 live births in 1993 to 114 per 1,000 live births in 2002, accompanied by a rise in the rate of CP from 44.4 to 100 cases per 1,000 live births in the same period.[2] A similar trend has been observed in Sweden and Western Australia.[1] In Indonesia, the higher survival rate of premature and other high-risk babies has also led to an increase of CP cases. High-risk babies are at risk of developing CP at a later age due to risk factors

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occurring in the pre-, peri-, and post-natal periods.

Cerebral palsy is a static, non-progressive disorder of motor and postural function due to an insult on the developing brain, which results in motor delays as well as postural and motion abnormalities.1 Some children with CP acquire various comorbidities and complications which may pose health threats and influence their quality of life.[3] Early detection of CP within the first year of life is essential to enable early intervention, which will affect the natural course of the disease.[4] Identification of CP in young infants is problematic due to the limited motor development in these infants, making it difficult to determine the types of motor delay that can be used to detect CP.4 Assessment of muscle tone and physiological reflexes, the cornerstones of CP diagnosis, are not always definitive.[4,5]

Several studies have reported methods for the early detection of CP before 3 to 6 months of age with reasonable predictive values, such as electroencephalography (EEG), cerebral function monitoring (CFM), brain magnetic resonance imaging (MRI) at 2 to 8 days of age, and spontaneous general movements (GMs) assessment at 2 to 4 months of age.[5-7] However, widespread use of such state-of-the-art methods is not feasible in developing countries such as Indonesia.

We aimed to determine the proportion of CP in high-risk babies, risk factors associated with CP, and the diagnostic performance of early detection methods using parameters of motor delay, physical examination, and cerebral ultrasound.

Methods

This prospective study followed a cohort of infants up to the age of 12 months. The study was done in Cipto Mangunkusumo Hospital, Jakarta, from April 2010 to July 2012. During the follow-up period, we performed bi-monthly motor development assessments and clinical-neurological examinations.

Using the appropriate formula, the minimum number of subjects required was calculated to be 150. Inclusion criteria were high-risk babies as signified by prematurity (gestational age of ≤32 weeks), low birth weight (2,4999 grams) and very low birth weight (≤1,500 grams), term or preterm neonates with meningitis, moderate or severe hypoxic-ischemic encephalopathy, intracerebral hemorrhage (ICH), and/or >48 hours of mechanical ventilation. We excluded infants with genetic, chromosomal, or metabolic anomalies, central nervous system malformations, neuromuscular disorders, and congenital infections. The independent variables were (1) the risk factors present; (2) cerebral ultrasound results; (3) motor delays; (4) primitive reflexes (palmar grasp, fisting, withdrawal, crossed-extensor, and traction response); and (5) postural reactions (protective-extension reflex and parachute reaction). The dependent variable was the occurrence of CP as determined by the gold standard examination of muscle tone and increased physiological reflexes at the specified ages.

At 4 to 5 months of age we performed the first motor development assessment and neurological examination comprising withdrawal reflex, palmar reflex, traction response, fisting, and crossed extensor reflex. At 6 months of age, motor development was again assessed, as well as all neurological examination items evaluated previously, with the addition of protective extension reflex. At 9 to 10 months of age, we followed up the subjects' motor development and performed all neurological examination items evaluated previously, with the addition of parachute reaction. The presence of CP was determined at the age of 6 and 12 months. The diagnosis of CP was made by one of two experienced pediatric neurologists when abnormalities in muscle tone and increased physiological reflexes were found, without evidence of regression or progression.

To determine the contribution of each respective risk factor and the diagnostic value of these predictors, we used data obtained at 4, 6, 10, and 12 months of age. As such, we determined the proportion of CP in high-risk babies and performed bivariate analyses on the potential association between risk factors and CP. We also determined the diagnostic performance of cerebral ultrasound to predict CP at 6 and 12 months of age, as well as the diagnostic values of motor delay and various clinical examinations done at 4, 6, and 9-10 months of age to predict CP at 6 and 12 months of age, respectively. A P value of <0.05 was considered to be statistically significant. After the study, we performed a Kappa test between the pediatric neurologist and one of our pediatric residents to assess inter-rater reliability to determine ease of

replication in daily medical practice. Kappa test was done in another group of 40 high-risk infants. The study protocol was approved by the Medical Research Ethics Committee of the University of Indonesia.

Results

During the study period, 178 high-risk babies visited our institution. Out of these, 150 fulfilled the criteria for analysis, while 28 infants were excluded (14 died and 14 were lost to follow-up due to undocumented address change). At 6 months of age, 39/150 subjects (26%) had CP, and at 12 months of age 36/150 subjects (24%) had CP. Diagnosis of CP was based on clinical manifestation. The majority of subjects were female (87%) and had gestational age <32 weeks (80%), birth weight <1,500 grams (75%), normal cerebral ultrasound (77%), as well as no history of meningitis (97%), intracranial hemorrhage (87%), or hypoxic-ischemic encephalopathy (HIE) (95%). On bivariate analysis, risk factors found to be associated with CP at the age of 6 and 12 months were cerebral ultrasound abnormalities, HIE, and ICH (**Table 1**). Gestational age was a significant predictor of CP at 12 months but not at 6 months of age. Sex, birth weight, meningitis, and duration of mechanical ventilation were not significantly associated with CP. Moderate and severe HIE were significant risk factors of CP, as were grade 3 and 4 IVH. Ultrasound abnormalities associated with CP included PVL, grade 3 and 4 IVH, encephalomalacia, meningitis, hydrocephalus, and ventriculomegaly.

The proportion of CP at 6 months of age was significantly higher in subjects with abnormal motor development and/or physical examination at 4 months of age than in those without. Odds ratios and diagnostic values of motor delay and other neurological examination parameters assessed at 4 months of age to predict CP at 6 months of age are presented in **Table 2**. Examination of palmar reflex, traction response (positive head lag) and fisting at 4 months had the best diagnostic value to predict CP at 6 months of age.

Subjects with motor delay and abnormalities on physical examination at 6 months of age had a

Table 1. Bivariate association between risk factors and cerebral palsy at 6 and 12 months

CP / Variables	At 6 months			At 12 months		
	OR	95%Ci	P value	OR	95%CI	P value
Gender Male Female	1.04 (reference)	0.60 to 1.82	0.87	1.22 (reference)	0.68 to 2.17	0.5
Gestational age ≤32 weeks >32 weeks	0.64 (reference)	0.36 to 1.13	0.14	0.5 (reference)	0.29 to 0.88	0.022
Berat lahir ≤1500 g >1500 g	1.8 (reference)	0.82 to 3.95	0.12	1.64 (reference)	0.74 to 3.62	0.20
Meningitis Yes No	0.76 (reference)	0.13 to 4.49	0.76	0.83 (reference)	0.14 to 4.89	0.83
Intracranial hemorrhage Yes No	4.31 (reference)	2.8 to 6.6	<0.001	4.49 (reference)	2.75 to 6.99	<0.001
Hypoxic-ischemic encephalopathy Yes No	4.47 (reference)	3.29 to 6.1	<0.001	4.91 (reference)	3.56 to 6.82	<0.001
Mechanical ventilation >48 hours <48 hours	1.12 (reference)	0.32 to 3.9	0.85	1.12 (reference)	0.32 to 3.92	0.84
Cerebral ultrasound Abnormal Normal	10.95 (reference)	5.77 to 20.8	<0.001	13.6 (reference)	6.54 to 28.35	<0.001

Table 2. Diagnostic values of parameters tested at 4 months to predict cerebral palsy at 6 months

Parameters	Cerebral palsy		Sensitivity, %	Specificity, %	OR	95%Ci	P value
	Yes	No					
Motor ability, n							
Abnormal	26	0	66.7	100			
Normal	13	113			9.6	9.43 to 10.2	<0.001
Withdrawal reflex, n							
Abnormal	25	0	64.1	100			
Normal	14	111			8.3	7.95 to 8.36	<0.001
Palmar reflex, n							
Abnormal	39	4	100	96.4			
Normal	0	107			0.03	0.01 to 0.09	<0.001
Traction response, n							
Abnormal	35	3	89.7	97.3			
Normal	4	108			12.21	4.12 to 36.2	<0.001
Fisting, n							
Abnormal	34	2	87.2	98.2			
Normal	5	109			26	9.10 to 50.92	<0.001
Crossed extensor reflex, n							
Abnormal	24	1	61.5	99			
Normal	15	110			7.4	4.94 to 12.95	<0.001

significantly higher proportion of CP at 12 months of age than normal subjects. Subjects with motor delay or abnormalities in primitive reflexes and protective extension reflex had higher risk of CP than those without. Motor delay and abnormal palmar reflex, fisting, traction response and protective extension had the best diagnostic value to predict CP at 12 months of age. Odds ratios and diagnostic values of motor delay and other neurological examination parameters assessed at 6 months of age to predict CP at 12 months of age are presented in **Table 3**.

We found a higher proportion of CP at 12 months of age in subjects who had motor delay or

Table 3. Diagnostic values of parameters tested at 6 months to predict cerebral palsy at 12 months

Parameters	Cerebral palsy		Sensitivity, %	Specificity, %	OR	95%Ci	P value
	Yes	No					
Motor ability, n							
Abnormal	35	7	97.2	93.8			
Normal	1	107			89	12.7 to 636	<0.001
Withdrawal reflex, n							
Abnormal	8	0	22.2	100			
Normal	28	114			4.2	3.64 to 7.1	<0.001
Palmar reflex, n							
Abnormal	33	2	91.6	98.2			
Normal	3	112			31.33	11.8 to 110.7	<0.001
Traction response, n							
Abnormal	25	1	69.4	99.1			
Normal	11	113			60	6.13 to 19.15	<0.001
Fisting, n							
Abnormal	34	2	94.4	98.2			
Normal	2	112			55.3	50.5 to 58.6	<0.001
Crossed extensor reflex, n							
Abnormal	13	113	36.1	8	0.1		
Normal	23	1				0.06 to 0.18	<0.001
Protective extension reflex, n							
Abnormal	35	8	97.2	92.9			
Normal	1	106			90	12.3 to 611.64	<0.001

abnormalities traction response, fisting, protective extension reaction, and parachute reaction at 9 to 10 months of age. Abnormalities in motor delay, primitive reflexes and postural reaction were associated with an increased risk of CP. Motor delay and postural reaction had the best diagnostic value to predict CP at 12 months of age. Odds ratios and diagnostic values of parameters assessed at 9 to 10 months of age to predict CP at 12 months of age are presented in **Table 4**.

At the age of 6 months, cerebral ultrasound had a sensitivity of 76.9%, specificity of 95.5%, positive predictive value of 85.7%, and negative predictive value of 92.2% for CP. At the age of 12 months, the abovementioned values for cerebral ultrasound were 74.3%, 94.6%, 82.8%, and 91.3%, respectively.

Kappa test was done between the pediatric neurologist and pediatric residents to assess inter-rater reliability for predicting CP at 6 and 12 months of age (with regards to exams for motor ability as well as primitive and developmental reflexes). Kappa test result was 0.9, indicating the ease and consistency of the methods for daily medical practice. The mean time taken to examine subjects' primitive reflexes at 4 months of age was 2 minutes and 37 seconds (SD 32.3 seconds), and the mean time taken to examine subjects' developmental reflexes at 9 months of age was 5 minutes and 18 (SD 53 seconds) seconds.

Discussion

The limitations of this study include the recruitment of subjects in a tertiary referral hospital, possibly leading to a higher proportion of CP than would be found in the general population, and follow-up largely done by home visits by only the principal investigator. However, this study has the advantage of being the first in Indonesia to determine the diagnostic value of clinical assessment results (motor ability as well as primitive and developmental reflexes) and cerebral ultrasound to predict CP in the first year of life.

The proportion of CP in our study was 26% at 6 months of age and 24% at 12 months of age.

Table 4. Diagnostic values of parameters tested at 9 to 10 months to predict cerebral palsy at 12 months

Parameters	Cerebral palsy		Sensitivity, %	Specificity, %	OR	95%Ci	P value
	Yes	No					
Motor ability, n							
Abnormal	36	7	100	93.8			
Normal	0	107			6.14	3.12 to 12.1	<0.001
Withdrawal reflex, n							
Abnormal	1	0	2.7	100			
Normal	35	114			3.2	2.18 to 9.23	0.074
Palmar reflex, n							
Abnormal	0	0					
Normal	36	114					
Traction response, n							
Abnormal	5	1	13.8	99.1			
Normal	31	113			3.9	2.8 to 4.6	0.001
Fisting, n							
Abnormal	3	1	8.3	99.1			
Normal	33	113			3.41	1.75 to 6.3	0.015
Crossed extensor reflex, n							
Abnormal	1	0	2.7	100			
Normal	35	114			3.33	3.18 to 5.68	0.074
Protective extension reflex, n							
Abnormal	25	2	69.4	98.2			
Normal	11	112			11.25	10.4 to 15.2	<0.001
Parachute reaction, n							
Abnormal	26	0	72.2	100			
Normal	10	114			12.5	11.4 to 14.15	<0.001

Our results were similar to those of Zafeiriou *et al.* who obtained an incidence of 28.5% in 204 high-risk babies.[8] The difference between the incidence at 6 and 12 months of age may be explained by the normalization of neurological features over time, possibly due to intervention, CNS maturation, or by improvement such features over time. Our results support the notion that clinical manifestations of CP can change with increasing age, particularly in the first year of life.[9]

We did not find a significant birth-weight- or gestational-age-specific difference in the incidence of CP. Our findings were in disagreement with literature stating that prematurity and low birth weight were risk factors for CP.[1,2] This finding may be due to improved perinatal health services and medical technology, enabling better hemodynamic monitoring leading to prevention of extreme fluctuations of cerebral blood flow, thus reducing the rate of complications such as ICH in infants born with a birth weight of 1,000-1,500 grams and infants born at gestational age of 28-32 weeks.[10] Only 30/150 subjects (20%) needed mechanical ventilation. Cools *et al.* reported that 90% of infants born at gestational age of <30 weeks required mechanical ventilation.[11] This difference may have been due to different gestational ages in the inclusion criteria or to advances in the management of premature babies, including surfactant therapy and the use of continuous positive airway pressure (CPAP), thereby reducing the need for mechanical ventilation.[12]

Cerebral ultrasound abnormalities were found in 35 subjects (23.3%). Six of these 35 subjects developed CP. There was a significant difference in the proportion of CP in infants with abnormal ultrasound compared to those with normal ultrasound ($P<0.001$). This result concurs with previous studies reporting that ultrasound abnormalities, especially grade 3 and 4 intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), and ventriculomegaly were associated with CP or other abnormalities of motor development.[13-15] All subjects with moderate and severe HIE (n=7) had CP, a significant difference from the proportion of CP in subjects with no or mild HIE ($P<0.001$). Similarly, previous studies reported that HIE, particularly in term infants, causes tissue damage in the form of PVL, focal and multifocal ischemia, and cerebral tissue necrosis.[16,17] Term infants made up the majority of subjects with HIE in our study (5/7). Forty-three out of 150 subjects (28.6%) had ICH; of these, 39.5% had CP. There was a significant difference in CP incidence in the ICH group compared to the non-ICH group, possibly due to the large proportion of grade 3 and 4 IVH found in the ICH group, which potentially develops into PVL cysts.[18]

On bivariate analysis, HIE, ICH, and ultrasound abnormalities showed significant associations with CP ($P<0.001$) at 6 and 12 months. Moderate and severe HIE were significant risk factors of CP, as were grade 3 and 4 IVH. Ultrasound abnormalities associated with CP included PVL, grade 3 and 4 IVH, encephalomalacia, meningitis, hydrocephalus, and ventriculomegaly, which were similar to previous findings in the published literature.[13-15]

The diagnosis of CP is straightforward in cases of severe CP or in older children. It is difficult to accurately diagnose CP in the first 6 months of life, in milder cases, or in cases of isolated motor delay. Motor delay is the first sign of CP and has a good sensitivity and specificity to detect CP after the age of 6 months, whereas neurological examination of primitive reflexes, postural reactions, and muscle tone as been reported to have a poor predictive value in the first months of life.[5]

In our study, when the subjects were evaluated at 4 months of age, all independent variables were significantly associated with the presence of CP at the age of 6 months. The most significant neurological features were palmar grasp reflex, traction response, and fisting. These results were in agreement with those reported by Morgan *et al.*[19] and the principal investigator's clinical experience. Motor delay did not yield a high OR, possibly due to infants' limited motor ability at 4 months of age. Variables measured during the evaluation at 4 months of age, especially palmar reflex, traction response, and fisting, had good sensitivity, specificity, and positive and negative predictive values in the range of 88 to 100%.

The evaluations done at the age of 6 months to predict CP at 12 months of age included all variables evaluated at 4 months and the protective extension reaction was added. All variables showed significant associations with later CP, with protective-extension reflex having the highest OR, followed by motor ability, traction response, fisting, and palmar grasp reflex. Motor ability and the protective-extension

reflex also had the highest ORs, as well as good sensitivity, specificity, and positive and negative predictive values. These two features can, therefore, be used as hallmarks for predicting CP at 12 months of age. Previous reports were in agreement with our results.[20] Infants who show poor postural control at 6 months of age need special attention and early intervention, as this skill is a prerequisite for sitting, standing, and walking.[20-22]

When a subject had a normal examination at 6 months of age, the child was re-evaluated at the age of 9 to 10 months. We found a higher risk of CP in subjects with abnormal protective-extension reflex, abnormal traction response, motor delay, fisting, and abnormal parachute reaction, compared to normal subjects. The primitive reflexes showed smaller ORs at 6 months than at 4 months. Motor delay showed lower ORs than were found at 4 and 6 months, but two examinations (motor delay and postural reaction showed superior sensitivity, specificity, when assessed at 9 to 10 months of age. As subjects progressed in age, the sensitivity of primitive reflexes tended to decline, although specificity remained high. After 6 months of age, primitive reflexes are no longer a sensitive tool and are rarely seen except in severe CP cases. Therefore, positive primitive reflexes at this age had high specificity for the diagnosis of CP. In mild cases, components of motor delay and postural examination can be used to diagnose CP. Results of this study confirmed the theory that postural reactions are a continuation of primitive reflexes, which reflect CNS maturation.[23] In practice, assessment of motor ability and postural reactions can be used to diagnose CP in infants older than 6 months.

All abnormalities found on cerebral ultrasound showed non-progressive anatomical lesions. Our results were in agreement with reports stating that causative insults produced static lesions in the brain.[12] However, the clinical manifestations of CP may change with advancing age, reflecting brain maturation and plasticity.[12] Cerebral ultrasound also had good sensitivity, specificity, and positive and negative predictive values, hence, it can be used in the early detection of CP.

In our study, neurological examinations such as for primitive and developmental reflexes were found to be simple, quick tests with high diagnostic value as tools to detect CP in the first year of life.

In conclusion, the proportion of subjects with CP at 6 months of age is 26% and at 12 months of age is 24%. In 88.7% of subjects, CP is detected in the first 6 months. Risk factors associated with CP at the age of 6 and 12 months are cerebral ultrasound abnormalities, HIE, and ICH. At 4 months of age, positive palmar grasp reflex, head lag, and fisting are predictive of CP at 6 months of age. Motor delay, positive palmar grasp reflex, positive head lag, fisting, and absent protective extension reflex at 6 months of age are predictive of CP at 12 months of age. At 9 to 10 months of age, motor delays, absent protective extension reflex, and negative parachute reaction were predictive of CP at 12 months of age. Cerebral ultrasound abnormalities were predictive of CP at 6 and 12 months of age. Kappa test result was 0.9 indicating the ease and consistency of the methods for daily medical practice.

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Conflict of interest

None declared

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Original Article

Factors affecting exclusive breastfeeding in term infants

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Abstract

Background Exclusive breastfeeding by healthy mothers to their healthy, term babies who underwent vaginal birth, should be readily accomplished. However, exclusive breastfeeding by Indonesian mothers has declined.

Objective To assess the monthly success rate prevalences for exclusive breastfeeding for the first 6 months of life, as well as factors that affect exclusive breastfeeding.

Methods A prospective cohort study was conducted in 243 healthy mothers with healthy term babies who underwent normal births at Cipto Mangunkusumo Hospital (CMH), Jakarta, Indonesia. Guided interviews were conducted monthly for six months. Bivariate and multivariate analyses were performed on the factors potentially affecting breastfeeding with equal subject numbers.

Results Exclusive breastfeeding prevalences were 64.8% (first month), 53.7% (second month), 43% (third month), 30.7% (fourth month), 23.5% (fifth month), and 22.3% (sixth month). Multivariate analysis revealed that the mother's confidence in breast milk production, as well as husband or family support, affected the success of exclusive breastfeeding for each month. Maternal not working/studying outside the home affected the success of exclusive breastfeeding in the third (RR 3.38; 95%CI 1.21 to 9.43) and fourth months (RR 6.56; 95%CI 1.39 to 30.99).

Conclusion Exclusive breastfeeding prevalences in Cipto Mangunkusumo Hospital decrease in a monthly fashion up to the sixth month. Several factors affect the success rate for each month in the six month period, including maternal confidence in breast milk production and family support. **[Paediatr Indones. 2018;58:25-35; doi: http://dx.doi.org/10.14238/pi58.1.2018.25-35]**.

Keywords: *exclusive breastfeeding; prevalence*

Breast milk is the most suitable nutritional source for an infant's needs up to the first 6 months of life. The benefits of breastfeeding are well known and include optimized infant growth and reduced death rates.[1,2] Healthy mothers and their healthy term babies who underwent vaginal births should not encounter many problems in exclusive breastfeeding. However, exclusive breastfeeding in Indonesia, according to the *Survei Demografi dan Kesehatan Indonesia (SDKI)* or *Demographic Survey and Indonesian Health Data* in 2007, was reported to be 43.9% in the first hour of life, 40.6% in infants up to four months old, and 32.4% in babies up to 6 months old.[3] The *Data Riset Kesehatan Dasar (RISKESDAS)* or *Basic Health Research Data* in 2010 by the Ministry of Health noted decreases to 29.3% in exclusive breastfeeding in the first hour of life, to 25.2% in infants up to 4 months old, and to 15.3% in infants up to 6 months old.[4] The decline in exclusive breastfeeding may be due to many factors,

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as reported by previous studies in developed and developing countries, such as care in the hospital or at home.[5-9] Studies on the success of exclusive breastfeeding have shown inconsistent results on the factors that affect breastfeeding in developed and developing countries.

In Indonesia, there have been few studies with a prospective design and monthly monitoring for 6 months on the factors that affect exclusive breastfeeding. As such, we aimed to assess the incidence rate and the factors affecting the success of exclusive breastfeeding monthly for 6 months, in healthy babies who underwent normal births at Cipto Mangunkusumo Hospital, the main referral hospital in Indonesia's health care system.

Methods

This study was a prospective, analytical, cohort study with an internal comparison group to evaluate the potential risk factors contributing to successful exclusive breastfeeding monthly for the first 6 months of life. The factors assessed were maternal educational level, early initiation, rooming-in care, education on breastfeeding techniques and benefits, avoiding early usage of feeding bottles, maternal psychological factors (confidence in breast milk production, husband/family support, as well as not having stress, confusion, fear, or post-natal depression), maternal work or studying outside the home, maternal physical factors (not sick or tired, not using contraceptives, not having an anatomical breast problem, breast engorgement, or cracked nipples), and indifference to formula milk promotions.

Subjects were recruited from the delivery ward of the Department of Obstetrics and Gynecology, Cipto Mangunkusumo Hospital, at the University of Indonesia Medical School, from December 2010 until June 2011 by consecutive sampling. The subjects were healthy mothers who gave normal per vaginam birth to term infants in Cipto Mangunkusumo Hospital. Their infants were considered to be healthy. We excluded infants with congenital malformations which prevented them from breastfeeding well, such as labiopalatognatoschisis, as well as infants with congenital heart disease, metabolic disease (as they were unable to receive breast milk, such as galactosemia, maple syrup urine disease, and phenylketonuria), twin infants, and mothers with human immunodeficiency virus (HIV).

Early subject samplings were conducted at the CMH rooming-in ward. Data acquisition then was conducted at the Growth and Development Clinic, Department of Child Health, University of Indonesia Medical School/Cipto Mangunkusumo Hosptial, by phone calls and monthly house visits, using guided interviews with a research questionnaire. The questionnaire had been tested previously in Child Health Department CMH.

Exclusive breastfeeding was defined as the feeding of breast milk only, without other liquids such as formula, orange juice, tea, honey, or plain water, as well as without additional food, such as banana, biscuits, or porridge, though administration of vitamins, minerals, and medicines were allowed.[1] Early initation was defined as the opportunity given to newborns to find the mother's breast and breastfeed. Early initiation was done as soon as possible after the birth. The infant was placed on the mother's chest with skin-to-skin contact. Early feeding may have included the use of nipples or milk bottles to administer breast milk or formula after the birth prior to 72 hours after the birth. Maternal working or attending school was defined as working or studying outside the home for more than 6 hours per day. Prevalences of infants who received exclusive breastfeeding every month were calculated based on subjects who stayed in the study until the end of that month. Subjects who dropped out were not included in the calculation for that month.

Variable analysis was done monthly for 6 months. Variables were analyzed using Chi-square hypothetical test to obtain P values, RR, and 95%CI for each month in the 6 months. Based on RR value, we calculated the population attributable risk (PAR) to evaluate the effect on the population if a certain factor was dismissed. For variables with insufficient sample sizes, results are given in a descriptive manner with text and tables.

The second analysis was multivariate logistic regression test. This analysis was used to assess factors that most affected the success of exclusive breastfeeding in each month for 6 months. The study protocol was approved by the Medical Research Ethics Committee of the University of Indonesia.

Results

The subjects' characteristics at the beginning of the study are shown in **Table 1**. The mean age of maternal subjects was 29.25 (SD 6.04) years, with the youngest and oldest aged 15 and 46 years, respectively. The mean infant birth weight was 3,041 (SD 3,520) grams.

Table 1. Subjects' characteristics

Characteristics	N=243
Maternal educational level, n (%)	
Low	75 (31)
High	168 (69)
Working mothers, n (%)	70 (29)
Infant gender, n (%)	126 (52)
Male	
Parity, n (%)	
Primipara	108 (44)
Multipara	135 (56)
Number of children, n (%)	
1	108 (44)
2	82 (34)
3	34 (14)
>4	19 (8)
Early initiation of breastfeeding, n (%)	97 (40)
Rooming-in, n (%)	241 (99)
Breastfeeding education, n (%)	136 (56)
Bottle use at hospital	6 (3)

The number of babies who received consistent, exclusive breastfeeding decreased in a monthly fashion for the 6 months studied. The largest decline occurred between the third and fourth months (29%). This declining trend is shown in **Figure 1**.

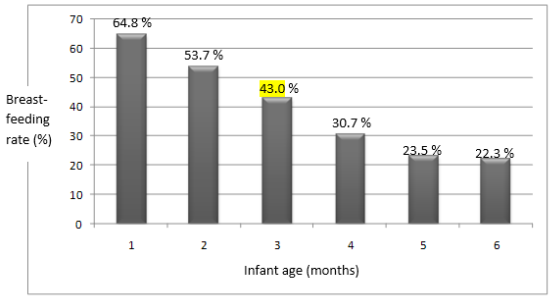

Figure 1. Prevalences of exclusive breastfeeding in months 1 - 6

Analysis of the factors potentially affecting the success of exclusive breastfeeding was done longitudinally for 6 months, revealing statistical and clinical differences. Complete explanations are presented in **Tables 2** and **Table 3**. Confidence in breast milk production and support form husband or family affecting the success of exclusive breastfeeding until 6 months. In population, if mothers was not confidence with her breast milk production and there was not support from her husband or family, exclusive breastfeeding will not accomplished 73-98%. Early initiation and stressed correlated with exclusive breastfeeding for 2 and 3 months, respectively. Not working or studying outside the home had affected the success of exclusive breastfeeding since 3rd months. A description of factors which affected the success of exclusive breastfeeding are shown in **Table 4**. More than 50% subjects were giving exclusive breastfeeding until 2nd months who were rooming in, avoided using milk bottle at hospital, not working/studying outside the home, not sicked/tired, not having inverted/flat nipple, and not interested in formula company promotion.

Multivariate analysis was conducted as a logistic binary regression with backward LR method. The analyses were done monthly for 6 months on factors which had P values <0.25, based on the bivariate analysis. Multivariate analysis results for the six months are shown in **Table 5**.

Discussion

The advantage of this study is that the "exclusive breastfeeding" and "non-exclusive breasfeeding" groups are from the same population and received similar observations with similar procedures. The observation on the success of exclusive breastfeeding and its influencing factors was performed monthly for 6 months, in order to limit recall bias. The questionnaire had been tested prior to the study, with the hope of increasing the reliability and validity of the study. Studies on factors affecting exclusive breastfeeding in term infants have been limited. Furthermore, there has never been such a study undertaken in Indonesia.

The main obstacle in our study was the difficulty of subject recruitment related to the ongoing renovation process at Cipto Mangunkusumo Hospital

Table 2. Statistical analysis results of factors affecting the success of exclusive breastfeeding

Variables	First month (n=239)			Second month (n=227)			Third month (n=216)		
	n (%)	PAR (%)	P value	n (%)	PAR (%)	P value	n (%)	PAR (%)	P value
High educational level	119 (67)	26	0.193	91 (55)	8	0.707	67 (42)	NA	0.750
Early initiation	74 (76)	37	0.002	57 (63)	25	0.028	39 (46)	39	0.499
Breastfeeding education	83 (62)	NA	0.287	65 (51)	NA	0.309	48 (40)	NA	0.257
Not working/ studying outside the home	-	-	-	-	-	-	87 (51)	81	0.001
Confidence in breast milk production	143 (82)	93	0.001	120 (70)	98	0.001	89 (60)	94	0.001
Support from husband or family	111 (82)	73	0.001	94 (75)	79	0.001	73 (60)	72	0.001
Not Stressed	136 (72)	71	0.001	110 (75)	67	0.001	85 (49)	70	0.001
Not using contraceptives	109 (63)	NA	0.442	42 (50)	NA	0.386	30 (45)	3	0.723
No cracked nipple/breast engorgement	89 (66)	8.7	0.567						

Variables	Fourth month (n=208)			Fifth month (n=204)			Sixth month (n=201)		
	n (%)	PAR (%)	P value	n (%)	PAR (%)	P value	n (%)	PAR (%)	P value
High educational level	47 (31)	4.4	0.856	36 (24)	9.4	0.726	33 (23)	3.5	1.000
Early initiation	28 (35)	11.5	0.343	21(27)	11	1.31	20 (26)	12	0.378
Breastfeeding education	34 (29)	NA	0.484	26 (22)	NA	0.610	25 (22)	NA	0.858
Not working/ studying outside the home	62 (39)	91	0.001	46 (30)	87	0.001	43 (28)	86	0.001
Confidence in breast milk production	63 (46)	98	0.001	47 (36)	96	0.001	44 (36)	96	0.001
Support from husband or family	58 (48)	87	0.001	45 (38)	90.3	0.001	44 (37)	97	0.001
Not Stressed	-	-	-	-	-	-	-	-	-
Not using contraceptives	15 (25)	NA	0.251	11 (19)	NA	0.69	10 (18)	NA	0.338
No cracked nipple/breast engorgement	-	-	-	-	-	-	-	-	-

NA=not available; (-)=descriptive analysis; PAR=population at risk

Table 3. Bivariate analysis result of factors which affect the success of exclusive breastfeeding

Variables	Fisrt month		Second month		Third month	
	RR	95%CI	RR	95%CI	RR	95%CI
High educational level	1.48	0.82 to 2.68	1.12	0.62 to 2.02	0.91	0.50 to 1.66
Early initiation	2.42	1.37 to 4.30	1.83	1.07 to 3.15	1.21	0.7 to 2.10
Breastfeeding education	0.75	0.44 to 1.28	0.76	0.45 to 1.29	0.73	0.42 to 1.26
Not working/ studying outside the home	-	-	-	-	6.48	2.61 to 16.13
Confidence in breast milk production	19.37	9.28 to 40.40	63.53	14.92 to 270.53	23.36	8.08 to 67.59
Support from husband or family	5.95	3.32 to 10.67	7.66	4.24 to 13.85	5.51	2.99 to 10.17
Not Stressed	3.98	2.07 to 7.66	3.50	1.68 to 7.28	3.90	1.70 to 8.91
Not using contraceptives	0.79	0.43 to 1.44	0.79	0.46 to 1.35	1.11	0.62 to 1.98
No cracked nipple/breast engorgement	1.17	0.69 to 1.99	-	-	-	-

Table 3. Bivariate analysis result of factors which affect the success of exclusive breastfeeding (continued)

Variables	Fourth month		Fifth month		Sixth month	
	RR	95%CI	RR	95%CI	RR	95%CI
High educational level	1.06	0.55 to 2.07	1.14	0.54 to 2.40	1.05	0.50 to 2.21
Early initiation	1.34	0.73 to 2.43	1.31	0.68 to 2.53	1.35	0.69 to 2.65
Breastfeeding education	0.81	0.45 to 1.46	0.84	0.44 to 1.62	0.94	0.48 to 1.84
Not working/ studying outside the home	14.09	3.3 to 60.16	9.92	2.31 to 42.55	8.99	2.09 to 38.67
Confidence in breast milk production	61.27	8.27 to 453.79	42.42	5.71 to 315.13	41.80	5.62 to 310.98
Support from husband or family	12.79	5.13 to 31.52	17.06	5.08 to 57.21	48.76	6.55 to 362.75
Not Stressed	-	-	-	-	-	-
Not using contraceptives	0.67	0.34 to 1.33	0.69	0.32 to 1.47	0.68	0.31 to 1.49
No cracked nipple/breast engorgement	-	-	-	-	-	-

NA=not available; (-)=descriptive analysis

Table 4. Descriptive factors which affects the success of exclusive breastfeeding

Variables	First month n (%)	Second month n (%)	Third month n (%)	Fourth month n (%)	Fifth month n (%)	Sixth month n (%)
Rooming-in care	154 (65)	121 (54)	92 (43)	64 (31)	48 (24)	45 (23)
Avoided using milk bottle at hospital	153 (66)	120 (54)	92 (44)	63 (31)	48 (24)	45 (23)
Not working/studying outside the home	155 (65)	119 (56)	*	*	*	*
Not sick/tired	154 (66)	121 (55)	93 (44)	64 (31)	48(24)	45 (22)
No inverted/flat nipple	152 (67)	120 (56)	92 (45)	63 (32)	47 (24)	44 (23)
No cracked nipple/breast engorgement	*	121 (55)	92 (43)	63 (30)	47 (24)	44 (22)
Not interested in formula company promotions	151 (67)	118 (55)	93 (46)	64 (33)	48 (25)	45 (24)
Not stressed	*	*	*	59 (33)	45 (25)	43 (25)

*=bivariate analysis

Table 5. Multivariate analysis of factors affecting the success of exclusive breastfeeding

Month	Variables	RR	95%CI	Probability (%)	P value
1	Husband/family support	4.06	2.06 to 8.03	89	0.001
	Confidence in breast milk production	14.85	6.90 to 31.94		0.001
2	Husband/family support	4.93	2.50 to 9.73	81	0.001
	Confidence in breast milk production	44.26	10.16 to 192.75		0.001
3	Not working mothers	3.38	1.21 to 9.43	69	0.020
	Husband/family support	2.35	1.15 to 4.81		0.019
	Confidence in breast milk production	14.62	4.88 to 43.83		0.001
4	Not working mothers	6.56	1.39 to 30.99	61	0.018
	Husband/family support	5.44	2.03 to 14.59		0.001
	Confidence in breast milk production	32.28	4.23 to 246.37		0.001
5	Husband/family support	6.49	1.77 to 23.75	21	0.005
	Confidence in breast milk production	19.85	2.573 to 153.16		0.004
6	Husband/family support	24.43	3.20 to 186.42	45	0.002
	Confidence in breast milk production	19.02	2.47 to 146.31		0.005

building. Other limitations were the possibility that the sample size of the control group was not equal to the risk factor-exposed group, due to its natural sampling formation. A large number of subjects (17%) dropped out of this study, probably due to its protracted duration. Factors expected to affect exclusive breastfeeding were attraction to formula milk promotions and maternal psychological state, such as confidence in breast milk production sufficiency, husband/family support, or not under stress. These factors being evaluated are subjective, time-dependent, and dynamic in nature.

In our study, 56% of mothers were multiparous, with an average of 2-3 children. Infant gender was almost evenly split between boys (52%) and girls (48%). The data obtained in this study was in accordance with the *2007 SDKI* data, which showed that more Indonesian women gave birth in their mid-20 to 30 years of age, and on average gave birth to 2.6 children in their lifetime. The gender distribution of Indonesia's population (49.6% males and 50.4% females) was also similar to that of our subjects.[3] A cross-sectional, community-based study in Tanzania reported that as many as 76% of mothers were multiparous with 2 as the median number of children. As many as 47% were males.[10] Similarly, a Malaysian cross-sectional study in 2009 reported 48.2% male infants and 51.8% female infants.[8]

In our study, the exclusive breastfeeding incidence in the 4th month was only 31%. This incidence was lower than the *2007 SDKI* data (40%), but higher than the *2002 SDKI* data (25%) or *RISKESDAS 2010* (26%). In the 6th month, only 22% were exclusively breastfeeding, lower than the *SDKI* data in 2002 (24%) and 2007 (32%), yet higher than *RISKESDAS 2010* (15%) for the same month.[3,4,11]

Other studies on exclusive breastfeeding for 6 months from several other developing countries in 2009 found higher percentages than our study. Studies from Malaysia, India, and Brazil showed 32.8%, 61.5%, and 31%, respectively, of infants received exclusive breastfeeding for 6 consecutive months.[7,8,12] These three studies used the same exclusive breastfeeding definition as our study, but different study methods.

The lower incidence of exclusive breastfeeding for 6 months in our study compared to the *SDKI* data and studies from other countries may be related to factors such as nutritional status of pregnant and lactating mothers, infant micronutrient status (iron, zinc, and vitamin A), and routine primary infant healthcare (growth evaluation and clinical signs of micronutrient deficiency).[13]

Seventy percent of the subjects had a high educational level, of senior high school or higher, yet educational level did not significantly affect exclusive breastfeeding. The median breastfeeding durations for mothers with high or low educational level did not differ (both at 2 months). This result was similar to that of other developing countries such as Ethiopia, although as much as 78% of subjects in their study stated that they had never attended school. Their multivariate analysis revealed no significant correlation between maternal educational level and the success of 6-month, exclusive breastfeeding ($P>0.05$).[14]

In contrast to studies from developing countries, those from developed countries found that educational level correlated to longer exclusive breastfeeding duration. For example, a Swiss study found that more than 60% of subjects had a high educational level (high school graduate minimum). This retrospective cohort study concluded that there was a significant correlation between level of education and the success of exclusive breastfeeding up to 6 months ($P<0.0001$). The median breastfeeding duration was 12 weeks for mothers with a high educational level and 7 weeks for mothers with a low educational level. The difference in median duration between the Swiss study and our study was due to a longer evaluation time conducted in our study.[9] We had hoped that increased knowledge and understanding about breastfeeding would result from a higher level of education. However, such did not appear to be the case. Nonetheless, we did not evaluate the level of knowledge and understanding on breastfeeding in our maternal subjects.

Early breastfeeding initiation in our study was only done in 99 subjects (40.7%), with 20 of these mothers succeeding in exclusive breastfeeding until the 6th month. This breastfeeding initiation percentage was lower than that of the *2007 SDKI* data (43.9%), yet higher than the *RISKESDAS 2010* data (29.3%).[3,4] *World Health Organization* data from 2007 also reported Indonesia's early breastfeeding initiation percentage at an almost similar rate of 39%. Compared to other Asian countries, we found a lower percentage than in Sri Lanka (75%) and the

Philippines (54%), but higher than in Nepal (31%) and India (24.5%).[15]

Cipto Mangunkusumo Hospital issued a policy stating that early breastfeeding initiation should be performed in the case of every healthy birth without contraindications. Therefore, the percentage should theoretically be 100%. Previous data for early breastfeeding in CMH was not available. The low percentage in our study may be caused by the fact that most initiations are not done according to procedure. For example, breastfeeding was not done directly after the birth, there was no skin-to-skin contact, and it was not up to the infant to decide when to breastfeed for the first time. The inadequate early initiation procedure may be due to limited human resources, lack of health officers' knowledge on correct initiation procedures or resistant behaviours.

In our study, early breastfeeding initiation affected the success of exclusive breastfeeding in the first two months of life. The lack of early initiation lowered the percentage of exclusive breastfeeding in the population 11-39% every month up to the 6th month, based on PAR calculations. Many studies in developing and developed countries also showed a greater impact of early initiation on breastfeeding success. Studies in India and Switzerland concluded that there was a significant correlation between early breastfeeding initiation and the success of exclusive breastfeeding (P value<0.0001).[6,16] The probability of infants who did not do early initiation to fail exclusive breastfeeding reached 55% (RR 1.2; 95%CI 1.08 to 1.34).[6] The different effect in our study compared to previous ones may be caused by different operational definitions.

Most mothers (241 subjects or 99%) opted for continuous rooming-in care. The other two subjects (1%) received rooming-in, post-natal care for only 12 hours, due to the lack of human resources to initially deliver the infant to the mother. Continuous rooming-in care is a CMH policy for mothers and infants who did not have contraindications. There is no time limit for initiation of rooming-in, so there were also infants who started rooming in at > 12 hours after birth. The longer the infant is separated from the mother, the greater the chance of the infant receiving formula milk. A Polish study concluded that infants who are separated for more than 1 hour from the mother within the first 24 hours of hospital care had a 3 times higher probability of receiving formula milk (OR 3.37; 95%CI 3.07 to 3.69).[5]

The percentage of exclusive breastfeeding success was higher in mothers with rooming-in care compared to non-rooming-in mothers for every month during the 6 months. From the 3rd month on, more mothers with rooming-in care failed to breastfeed exclusively. This observation indicates that rooming-in care played a role in the success for the first 2 months. Several obstacles which might cause mothers to refuse rooming-in care were an ill or tired condition, no family available to assist the mother, or the lack of healthcare personnel to help.

Studies in developing and developed countries have reported a correlation between rooming-in care and successful exclusive breastfeeding. A study in Brazil found that mothers with rooming-in care had a 35-times higher probability to successfully exclusively breastfeed up to the sixth month (P=0.0297).[12] In addition, a Swiss study concluded that 55% (OR 1.21; 95%CI 1.09 to 1.33) of mothers who did not have rooming-in care failed to exclusively breastfeed up to the sixth month.[6]

In our study, 136 subjects (56%) received education on breastfeeding techniques and benefits. However, we found that education did not correlate with successful exclusive breastfeeding monthly for 6 months. The PAR for each month in the 6 months for this factor could not be calculated due to RR values being less than 1.

A prospective, random, clinical trial in Singapore concluded that a one-time, structured and personal session on breastfeeding with a lactation counselor increased the success of exclusive breastfeeding until the 3rd month (P<0.0001), but not at the 6th month (P>0.05).[17]

In our study, breastfeeding education was given by nurses or nursing students. Subjects received verbal information on breastfeeding techniques and benefits, without display exhibits. Sessions lasted for 10-15 minutes, were performed post-birth in the ward room and consisted of 6 mothers and 2 educators. The lack of correlation between education and successful, exclusive breastfeeding may be due to inadequate methodology, duration, consistency, or persistency of the educational session.

In our study, 3% of infants used bottles while at the hospital. The bottle was intended for giving

either expressed milk (2 subjects) or formula milk (4 subjects). A Swiss, restrospective, cohort study reported that 58% of infants who used a bottle in the first week failed to receive exclusive breastfeeding (P<0.0001; OR 1.38; 95%CI 1.25 to 1.52).[18] A Brazilian, epidemiological study reported that bottle usage in the first week was 56% and increased to 74% in the first month. Also, bottle usage in the first week of life was a risk factor for failure to exclusively breastfeed (OR 4.01; 95%CI 2.07 to 7.78).[19]

Bottle usage at CMH is not advised unless there is a condition for which the mother cannot breastfeed directly. As such, expressed breast milk may be given by cup feeding. The effect of bottle usage can cause nipple confusion and indolence due to the different shape and contour of bottle nipple from the mother's nipple. The first days of an infant's experience play an important role, hence, earlier bottle usage may lead to shorter duration of exclusive breastfeeding.[18,19]

Psychological factors, including maternal confidence in sufficient breastmilk production and support from husband/family had a statistically significant correlation with successful exclusive breastfeeding monthly for the first 6 months (P=0.001). Mothers who firmly believed that their breastmilk production was sufficient had a 19-66 times higher probability of successful exclusive breastfeeding monthly compared to mothers who lacked that confidence. In the population, the lack of belief in sufficient breastmilk production reduced the success of exclusive breastfeeding by 93-98% per month.

A prospective cohort study in California and Connecticut, USA, showed similar findings as our study. The California study reported that mothers who were not sure of their breast milk production were 2.8 times more likely to fail to exclusively breastfeed in the second month (95%CI 1.02 to 7.6) and 1.2 times in the third month (95%CI 0.84 to 1.7) compared to mothers who were confident of their breast milk production.[9] The Connecticut study reported that 28% of mothers felt unsure of their breast milk production in the second week after birth. The unsure mothers had a 3 times higher chance of failing to exclusively breastfeed in the second week (95%CI 1.64 to 4.79; P<0.0001), and 12 times higher in the second month (95%CI 1.79 to 76.5; P<0.001) compared to mothers with a firm belief in their breast milk production.[20]

In our study, mothers with support from their husbands or families had a 6-49 times higher likelihood to successfully breastfeed exclusively for every month in the 6 months compared to mothers with no support. In our patient population, lack of husband/family support reduced exclusive breastfeeding success as much as 73% in the first month, 79% in the second month, 72% in the third month, 87% in the fourth month, 90% in the fifth month, and 97% in the sixth month.

Similarly, a California study reported that a husband's support affected the success of exclusive breastfeeding in the second week, while mothers without spousal support were twice as likely to fail. A difference in their study was their smaller OR compared to ours, as only 77% of their subjects were married, while 100% of our subjects were married.[9]

Another psychological factor analyzed in our study was maternal feelings, which did not significantly correlate to the success of exclusive breastfeeding until the third month (P=0.001). Mothers who did not feel stressed, confused, worried, afraid, or depressed had a 4 times higher likelihood to succeed in exclusively breastfeeding to 3 months (RR 3.50 to 3.98). This was the most influential factor for the success of the first month of breastfeeding (RR 3.98; 95%CI 2.07 to 7.66). The PAR results showed that in our patient population, mothers who felt stressed, confused, worried, afraid, and depressed had reduced success in exclusively breastfeeding to 71% in the first month, 67% in the second month, and 70% in the third month.

In terms of depression, a California study showed similar effects to ours, that depression is one of the factor. They evaluated 239 mothers (24%) with post-birth depression based on the *Center for Epidemiologic Studies Depression Scale* (CES-D). Mothers with high CES-D scores (>16) in the first 2 weeks had a 1.2 times higher likelihood of failing to exclusively breastfeed until 3 months (P=0.01), compared to mothers who were not depressed.[9] The difference between the California study with this study was in the method to evaluate depression. Another US study concluded that only 9.7% of mothers with emotional dysfunction could breastfeed exclusively for 6 months (OR 0.53; 95%CI 0.31 to 0.92).[21]

Four physical maternal factors were evaluated: cracked nipple or breast engorgement, maternal illness

or fatigue, breast anatomical problems, and hormonal contraceptive usage. The percentage of exclusive breastfeeding success in the first month was higher in mothers who did not experience cracked nipples or breast engorgement, but it was not statistically significant. In our subjects, cracked nipple or breast engorgement caused a decrease in breastfeeding success of 9% in the first month. No analyses were performed for the 2nd to 6th months, due to the small number of mothers who experienced cracked nipples or breast engorgement compared to the sample size calculation. In the 2nd month, mothers without cracked nipples or breast engorgement were more successful in exclusive breastfeeding (56%), but not in the 3rd to 6th months. In the 6th month, there were two mothers with cracked nipples or breast engorgement caused by incorrect latching on by their infants. The percentage of exclusive breastfeeding success was higher in mothers who did not feel ill or tired, with 66% in the 1st month and 55% in the 2nd month. In the 4th to 6th months, we did not find mothers who felt ill or tired.

Similarly, Taveras *et al.* reported that maternal physical condition, such as cracked nipples, breast engorgement, illness, or fatigue affected the success of exclusive breastfeeding in the 2nd week (P=0.001) and in the 3rd month (P=0.03). However, their multivariate analysis determined that maternal physical condition only affected exclusive breastfeeding success significantly in the 2nd week (OR 1.5; 95%CI 1.1 to 1.97), but not in the 3rd month (OR 1.2; 95%CI 0.94 to 1.6).[9]

Mothers without inverted or flat nipples in our study were more successful in exclusively breastfeeding than with inverted/flat nipples in the 1st month (152 subjects) and 2nd month (120 subjects). Three subjects with inverted or flat nipples successfully breastfed exclusively in the 1st month using nipple connectors. At the end of observation, there was 1 subject with a flat nipple who succeeded in breastfeeding exclusively for 6 months.

A cross-sectional study in Tanzania reported a significant correlation between mothers without anatomical nipple disorders and the success of exclusive breastfeeding for 6 months (P<0.0001). Their multivariate analysis showed that mothers without anatomical nipple problems had a 7 times higher likelihood of successful exclusive breastfeeding for 6 months compared to mothers with an anatomical disorder (OR 6.6; 95%CI 3.2 to 13.6).[10]

Maternal contraceptive usage did not significantly correlate to exclusive breastfeeding monthly for the 6 months of our study. Most subjects used contraceptives by the 40th to 60th day after birth. In the 2nd month after birth, all subjects who failed to breastfeed exclusively were also using hormonal contraceptives (56%). Similarly, a prospective cohort study in Germany concluded that there was no statistically significant difference in exclusive breastfeeding between with and without contraceptive groups. However, 25% of mothers in the hormonal contraceptive group failed to breastfeed exclusively with the excuse of diminished milk production, while only 13% of mothers in the non-hormonal contraceptive group failed to breastfeed exclusively.[22]

In our study, 72% of mothers did not work outside the home. Not working significantly affected the success of exclusively breastfeeding in the 3rd month and did so consistently until the 6th month. (P=0.001-0.022). Non-working or schooling mothers had a 6-14 times higher likelihood of exclusively breastfeeding in the 3rd to 6th months, compared to working or schooling mothers. The possibility of success fluctuated monthly with the highest relative risk in the 4th month (RR 14.09; 95%CI 3.3 to 60.16). In population, maternal working/studying outside the home caused failure to exclusively breastfeed by as much as 82% in the 3rd month, 91% in the 4th month, 87% in the 5th month, and 86% in the 6th month (PAR).

Several studies found a correlation between maternal working and successful exclusive breastfeeding. A cross-sectional study in Klang, Malaysia, concluded that working mothers had an almost 4 times higher likelihood of failing to breastfeed exclusively for 6 months compared to non-working mothers (OR 3.75; 95%CI 1.64 to 8.55).[8] A similar study on nursing behavior and exclusive breastfeeding in Singapore concluded that working status affected the success of exclusive breastfeeding (HR 1.27; 95%CI 1.14 to 1.41).[23] A study from another developed country, USA, also found that mothers who returned to work or study had an almost 3 times higher possibility to fail to exclusively breastfeed compared to non-working mothers (RR 2.4; 95%CI 1.75 to 3.3).[9]

In our study, most working mothers returned to work or study after their 2 month maternity leave, but a small number returned after only 1 month of leave. Based on the Indonesian human resources law no. 13, article 82 verse 1 (2003), the length of maternity leave is at least 1.5 months before birth and 1.5 months after birth, based on doctor's or midwife's calculated due date.[24] Mothers who returned to work or study typically started supplementing with formula beginning in the second month as preparation for returning to work In our study, mother's working status significantly affected the success of exclusive breastfeeding starting from the third month. Going back to work was the most common reason for mothers to stop exclusive breastfeeding. Most subjects were unaware of how to express milk and store it. Lack of support and commitment at the workplace or school also caused failure to breastfeed exclusively.

Exclusive breastfeeding success for 6 months was higher in mothers who were not interested in formula company promotions. More mothers who were not interested in formula promotions successfully breastfed exclusively in the 1st month (151 subjects) and the 2nd month (118 subjects). However, the number of mothers not interested in formula promotions declined consistently with time.

A multicenter study in Ethiopia on 1,142 mothers with 6-month-old infants reported that 44.1% of subjects admitted being interested in formula milk promotions on television, radio, and newspapers, but there was no significant correlation with failure to exclusively breastfeed ($P>0.05$).[14]

In our study, the effect of formula promotion was observed after the 2nd month post-partum, along with improved maternal physical and psychological well-being. In addition, maternal indifference to formula promotion may also be affected by socioeconomic factors.

Logistic regression analysis, which included all factors, showed that the success of exclusively breastfeeding by month was influenced by many factors. The probability of success by month may be estimated from data relating to factors contributing to the success of exclusive breastfeeding in a multivariate manner.

Healthy mothers with healthy, term infants, confidence in breast milk production and who received support from husbands/families, had probabilities of 89% and 81% in the 1st and 2nd months, respectively, for successful exclusive breastfeeding. The probability for successful exclusive breastfeeding in the 3rd and 4th months were 69% and 61%, respectively, for mothers with confidence in their breast milk production, had husband/family support, and did not work or study outside the home. The probability of successful exclusive breastfeeding in the 5th and 6th months were 21% and 45%, respectively, for mothers with confidence in their breast milk production and husband/family support.

In conclusion, prevalences of exclusive breastfeeding in healthy term babies who had normal births in CMH decrease in a monthly fashion. After eliminating bias, maternal confidence in breast milk production and husband/family support are found to most influence the success of 6 months exclusive breastfeeding. Not working/studying outside the home also influence the success rates for the 3rd and 4th months.

Conflict of Interest

None declared.

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Original Article

Sleep disorders in children with attention-deficit/hyperactivity disorder

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Abstract

Background Chemotherapy-induced nausea and vomiting are some Background Attention-deficit/hyperactivity disorder (ADHD) is a neurobehavioral abnormality that commonly occurs among children. Sleep disorders are comorbid with ADHD. Sleep disorders in Indonesian children with ADHD have not been widely studied.

Objective To understand the proportion and factors that influence sleep disorders in children with ADHD.

Methods This cross-sectional study involved 54 children aged 3-14 years who had been diagnosed with ADHD by a pediatric growth and development consultant using DSM-5 criteria. The subjects were consecutively selected from March to August 2017 at the Child Development Polyclinic, Dr. Sardjito Hospital, Yogyakarta. Sleep data were collected using the *Sleep Disturbances Scale for Children* (SDSC) and the *Children's Sleep Hygiene Scale* (CSHS).

Results Of the 54 children with ADHD (46 males and 8 females), 35 (64.8%) experienced sleep disorders. The majority (26 subjects, 48.1%) had the disorder of initiating and maintaining sleep. Children with the combined (inattention and hyperactive-impulsive) type of ADHD experienced significantly greater sleep disturbance compared to the inattention type or hyperactive-impulsive type (OR=3.750; 95% CI 1.133 to 12.41; P=0.027). Poor sleep hygiene was also significantly associated with more severe sleep disorders (r=-0.383, P=0.004).

Conclusion The proportion of sleep disorder in children with ADHD is relatively high, with the majority having a disorder of initiating and maintaining sleep. Children with combined type ADHD experience a higher amount of sleep disorder than those with either the inattention or hyperactive-impulsive types of ADHD. Children with poor sleep hygiene have significantly more severe sleep disorders. **[Paediatr Indones. 2018;58:48-52; doi: http://dx.doi.org/10.14238/pi58.1.2018.48-52]**.

Keywords: *sleep disturbance; ADHD; SDSC; CSHS*

Sleep is a basic human necessity. Quality of sleep impacts the quality of life in children. Various psychiatric, somatic, and neurologic diseases often follow sleep disorders. A number of neurological diseases are linked with sleep disorder including Asperger's syndrome, Tourette's syndrome, ADHD, autism, epilepsy and learning/motoric difficulties.[1]

Attention-deficit/hyperactivity disorder (ADHD), a neurobehavioral abnormality, is a chronic condition most frequently affecting school-aged children.[2] The prevalence of ADHD is 5% amongst children worldwide.[3] However, Gamayanti et al. found the prevalence of ADHD children in Yogyakarta to be 6.68%.[4] Very little study has been done on the prevalence of ADHD in Indonesia. Common comorbidities with ADHD include learning difficulty (15-20%), sleep disturbance (50-80%), obesity, language difficulty (30-35%), anxiety (20-25%), mood disturbance (15-20%), conduct disorder (20%), oppositional defiant disorder (40%) and substance abuse (15%).[5] Sleep disorders are reported

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by 25-50% of parents of children and teenagers with ADHD, especially with regards to their children's difficulty initiating and maintaining sleep.[6] Sleep problems in children with ADHD are linked with worsened daily function and intensified ADHD symptoms.[7] Factors that contribute to sleep disorders in ADHD include circadian rhythm irregularity, poor sleep hygiene, delinquency, anxiety and mood disturbance, as well as use of stimulant drugs.[8]

We aimed to understand the proportion of sleep disorders and also factors that influenced sleep disorders in children with ADHD. As such, we hope our study results will be of benefit towards providing clear explanations for parents regarding factors that influence sleep disorders in children with ADHD. Thereby children with ADHD may be able to receive comprehensive management so as to prevent medical and social consequences.

Methods

This analytic, cross-sectional study was conducted in the Child Development Polyclinic, Dr. Sardjito Hospital, Yogyakarta, from March to August 2017. We utilized primary data from the SDSC questionnaires that had been filled based on history of sleep patterns history in the last six months, and the CSHS questionnaire, based on the sleep hygiene history of the past month.

The SDSC had been validated against 147 children aged 5-16 years and obtained the value of Cronbach's alpha of 0.71 to 0.79.9 In Indonesia, the results of Natalita et al. study showed that the diagnostic results of SDSC on wrist actigraphy obtained 71.4% sensitivity and 54.5% specificity, with positive predictive value and negative predictive value respectively 75% and 50%, so this instrument can be used as a screening tool for sleep disorders.[10]

Validity test was done by testing the questionnaires to 30 respondents which has the same characteristics at the Child Development Polyclinic, Dr. Sardjito Hospital, Yogyakarta. Using Pearson product moment correlation, the result of validity test was valid (r count > 0.361). For reliability test, we get the value of Cronbach's alpha was 0.749.

Subject recruiutment was done through consecutive sampling. Inclusion criteria were children that had been diagnosed with ADHD by a pediatric growth and development consultant using the DSM-5 criteria,[2] in good general health, aged 3-14 years, and whose parents were willing to be respondents. The exclusion criteria were children with illnesses that affected their sleep pattern, namely, uncontrolled asthma.

Bivariate statistical analysis was carried out using the Chi-square method to analyze relationships between dependent variables [ADHD types and medication (methylphenidate)]. Possible correlation between sleep hygiene and sleep disorder was analyzed by Pearson's test. This study was approved by the Research Ethics Committee at Dr. Sardjito Hospital.

Results

During the study period, 58 children aged 3-14 years were diagnosed with ADHD. Among them, four subjects with asthma were excluded from the study. Hence, 54 children who fulfilled the inclusion criteria. The subjects' characteristics are presented in **Table 1**.

The proportion of subjects with sleep disorders was relatively high (35 subjects, 64.8%). Types of sleep disorders based on the SDSC questionnaire are presented in **Table 2**. The most common form of sleep disorder found in this study was initiating and maintaining sleep (26 subjects, 48.1%), followed by sleep-wake transtion disorder (8 subjects, 14.8%) and excessive somnolence disorder (1 subject, 1.9%).

The analysis results of subgroup and bivariate variables associated with sleep disorders are summarized in **Table 3**. Chi-square test revealed that children with combined type ADHD had 3.75 times increased risk of sleep disturbance than inattention and hyperactive-impulsive types of ADHD (OR 3.750; CI 1.133 to 12.41; P=0.027). Medication for ADHD was not significantly associated with sleep disorders in children with ADHD.

Pearson's correlation coefficient was used to analyzed the relationship between sleep hygiene and sleep disorders. The r-value (correlation coefficient) was -0.383, with P value = 0.004. The negative correlation coefficient indicated that in children with ADHD, poorer sleep hygiene was significantly associated with more severe sleep disorders, with both variables having a significant connection.

Table 1. Research subjects' characteristics

Characteristics	(N=54)
Age, n (%)	
3-6 years	28 (51.9)
≥7 years	26 (48.1)
Sex, n (%)	
Male	46 (85.2)
Female	8 (14.8)
ADHD type, n (%)	
Inattention	14 (25.9)
Hyperactive-impulsive	4 (7.4)
Combined	36 (66.7)
Stimulant drugs, n (%)	
Yes	11 (20.4)
No	43 (79.6)
Sleep hygiene, n (%)	
Good	29 (53.7)
Poor	25 (46.3)

Table 2. Types of sleep disorder in children with ADHD

SDSC results	(N=54)
No sleep disorder, n (%)	19 (35.2)
Sleep disorder, n (%)	
Disorder of initiating and maintaining sleep	26 (48.1)
Sleep-wake transition disorder	8 (14.8)
Excessive somnolence disorder	1 (1.9)
Total	35 (64.8)

male counterparts.[12]

Sleep disorders occured in 64.8% of the children with ADHD, with a total of the score SDSC exceeding 39. The proportion of sleep disorders in our subjects was slightly less than that of a study conducted in the Child Development Polyclinic of Sanglah Hospital, Bali, which also used the SDSC questionnaire, though there were differences in subjects' age range and population. Wedayanti reported that in subjects with an age range of 7-12 years, sleep disorders occured in 22 (66.7%) children with ADHD, the majority being male respondents (25 subjetcs, 75.8% of all ADHD subjects).[13]

The disorder of initiating and maintaining sleep includes duration of sleep at night, time needed for the child to fall asleep after going to bed, resisting sleep, difficulty sleeping at night, fearing sleep, awakening from sleep more than twice nightly, and difficulty going back to sleep once awake. From the SDSC assessment, 35 (64.8%) subjects experienced sleep disturbances, in with 26 (48.1%) subjects having a disorder of initiating and maintaining, needing 15-30 minutes to sleep since going to bed, and occasionally (1-2 times per week) the children were unwilling and rejected sleep.

Table 3. Bivariate analysis of ADHD type and medication with sleep disorders

Variables	Sleep disorder (n=35)	No sleep disorder n=19)	P value	OR (95% CI)
ADHD type, n(%)				
Combined	27 (75)	9 (25)	0.027	3.750 (1.33 to 12.41)
Inattention or yyperactive-impulsive	8 (44.4)	10 (55.6)		
Stimulant medication,* n (%)				
Yes	7 (63.6)	4 (36.4)	0.927	0.938 (0.236 to 3.724)
No	28 (65.1)	15 (34.9)		

P-value <0.05 was considered statistically significant; (Chi-square and *Fisher's tests)

Discussion

In this study, the proportion of ADHD among 3 to 6-year-olds and ≥7-year-olds did not widely differ. However, there were more male than female subjects. Based on 19 reviewed studies, the ADHD prevalence was estimated to be 5-10% of school-aged children.[11] The estimated male to female ratio was is 3:1 in a population-based study, and 5:1 to 9:1 in clinical samples. Female children with ADHD are reported to have fewer hyperactive-impulsive symptoms and more inattention symptoms when compared to their

LeBourgeois *et al.*, conducted a cross-sectional study on 45 ADHD children aged 6-19 years. They found no distinction between sleep disorders and ADHD subtypes.[14] Mayes *et al.*, carried out a study to understand the link between ADHD subtype and sleep disorders using the P*ediatric Behaviour Scale* (PBS) questionnaire on ADHD children between 6-16 years of age (normal IQ ≥80), which included 271 mixed type and 144 inattention type ADHD children. They reported that mixed type ADHD was more linked to sleep disorders than inattention type ADHD, even though children with inattention type

ADHD experienced frequent afternoon sleepiness.[15] However, in Taiwan, Chiang *et al.* conducted a study on 325 children with ADHD aged 10-17 years using the *Sleep Disturbance* and found no significant difference in subtypes and sleep disorders between combined type and inattention type ADHD.[16] In addition, Yoon *et al.* compared sleep quality in adults aged 19-62 years with combined type ADHD and inattention type ADHD. They used the *Epworth Sleepiness Scale, Pittsburgh Sleep Quality Index,* and *Fatigue Severity Scale*. Chi-square, Mann-Whitney U test and MANOVA analyses indicated that subjects with inattention type ADHD had a poorer quality of sleep and fatigue in compared to those with combined type ADHD.[17]

Methylphenidate is a psychostimulant class drug that is used to treat children with ADHD. We found that medication was not significantly associated with sleep disturbance (P=0.927). But the lack of correlation was probably due to the small number of subjects taking stimulant drugs. We also did not review dosage and length of administered therapy as variables. Parents of children who received stimulant drugs reported a high prevalence of sleep disorder (29%) and complaints of difficulty sleeping at night or insomnia, potentially aggravating the ADHD symptoms.18 A cross-sectional study using the *Sleep Behavior Questionnaire and Child Behavior Checklist* was done to compare children with ADHD who had received stimulant drugs and those who had not. In 142 children aged 4-18 years, more children who received stimulant drugs experienced severe sleep disorders compared to those who did not receive therapy (29% *vs.* 10%, respectively).[19]

Sleep hygiene is the adjustment of various behaviors and environments that influence the process of initiating and maintaining sleep. Many children with ADHD tend to encounter difficulty achieving adequate sleep habits, such as forming daily routines, regular sleep schedule, and regular waking times.[20] Restoring sleep hygiene steps has been proven to improve quality of sleep and effectively resolve sleep disorders in ADHD children. Sleep hygiene involves developing consistent behavior around bedtime to enhance productive and comfortable sleep. In children, factors that may improve sleep include calm sleep routines and regular sleep and wake times. Of these interventions, the most important one is a regular waking shedule.[21] Behavioral practices to shape good sleep hygiene (adapted from the *American Academy of Sleep Medicine*, 2008), include: organizing regular and consistent sleep schedules, relaxing sleep routines, creating comfortable bedroom setting, using the bedroom for sleep only, minimizing before-bed activities and electronic use, avoiding emotionally disruptive conversations and activities, not consuming caffeine less than 6 hours before sleep, structuring a consistent wake time, avoiding afternoon naps, exercising during the day or afternoon, relaxing activities (such as yoga) before bed, as well as avoiding drugs that may interfere with sleep (such as decongestants and certain asthma medication).[21]

In conclusion, the proportion of sleep disorders in children with ADHD is relatively high, at 64.8%. The most common sleep disorder is initiating and maintaining sleep (48.1%). Children with combined type ADHD and poor sleep hygiene have a higher risk of sleep disorders. We found no correlation between use of stimulant medication and sleep disorders in children with ADHD. In children diagnosed with ADHD, sleep disorder assessments are needed to allow for earlier management. Education and sleep hygiene remain the initial therapies for tackling sleep disturbances in children with ADHD.

Conflict of Interest

None declared.

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Original Article

Obesity and functional constipation in children

Natasha Yuwanita, Atan Baas Sinuhaji, Tiangsa Sembiring, Supriatmo, Ade Rachmat Yudiyanto

Abstract

Background Functional constipation is a common pediatric problem in both developed and developing countries. In the past two decades, the prevalence of obesity has increased worldwide. Obesity itself leads to many health problems, including functional constipation. Studies correlating obesity to functional constipation have thus far mostly originated from developed countries.

Objective To assess for a possible correlation between obesity and functional constipation in children in a developing country.

Methods This cross-sectional study was conducted in Al-Mukhlisin Islamic Boarding School, Batu Bara District, North Sumatera Province, Indonesia, between July and August 2015. The subjects were 150 students aged 12 to 17 years. Questionnaires were used to determine functional constipation and filled by direct interview. Obesity was determined by body mass index. Data were analyzed using Chi-square test.

Results Of 150 children, 49 had functional constipation; and 18 of the 49 were obese. The mean age of children with constipation was 14.7 (SD 1.07) years (95%CI 14.1 to 14.7) and their mean body weight was 53.8 (SD 15.10) kg (95%CI 49.4 to 58.1). The prevalence for functional constipation in obese children was 58% There was a statistically significant correlation between obesity and functional constipation (prevalence ratio=4; 95%CI 1.72 to 8.94; P=0.001), indicating that obese children had 4 times higher risk of having functional constipation.

Conclusion There is a significant correlation between obesity and functional constipation in children. **[Paediatr Indones. 2018;58:1-4 ; doi: http://dx.doi.org/10.14238/pi58.1.2018.1-4]**.

Keywords: *obesity; functional constipation; prevalence; children; developing country*

Functional constipation is one of the most common gastrointestinal problems in children, with around 3% of all pediatric cases. In the past two decades, the obesity prevalence has also increased.[1] Most studies on a correlation between obesity and functional constipation have been done in developed countries.[2] Obese children usually consume a low fiber diet and engage in less physical activity than normoweight children. These two factors result in altered defecation patterns.[3]

A recent pediatric study reported a significantly higher prevalence of obesity in children with functional constipation (23%) compared to a control group.[4] In an effort to increase children's quality of life, we aimed to assess for a possible correlation between obesity and functional constipation.

This study was presented at the 5th Global Congress for Consensus in Pediatrics and Child Health, Xi'an, China, March 3-6, 2016.

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Methods

This cross-sectional study was done to examine a potential correlation between obesity and functional constipation in children aged 12 to 17 years. Participants were recruited by consecutive sampling from the Al-Mukhlisin Boarding School at Batu Bara District, North Sumatra Province from July to August 2015.

Subjects were generally healthy at the time of investigation and had no chronic or acute illnesses. Children were excluded if they were malnourished or overweight, had gastrointestinal or enodcrine disorders, diarrhea, vomiting, fever, failure to thrive, blood in the stool, or organic abnormalities. This study was approved by the Ethics Committee for Research, University of Sumatera Utara Medical School. Subjects were included after obtaining informed consent from their parents or guardians.

The subjects were surveyed by questionnaire and direct interview to assess the incidence of functional constipation, based on ROME III criteria. The criteria consisted of two or fewer defecations in the toilet per week, at least one episode of fecal incontinence per week, history of retentive posturing or excessive volitional stool retention, history of painful or hard bowel movements, presence of a large fecal mass in the rectum, and history of large diameter stools which may obstruct the toilet. A diagnosis of functional constipation was made when these criteria were fulfilled at least two of them, once per week, for at least 2 months prior to diagnosis.

All participants underwent physical examinations performed by a physician. Body height (BH) was measured to the nearest 0.5 cm using a portable stadiometer (*Microtoa 2 M*). Body weight (BW) was measured on a *Camry Scale* with a precision of 0.5 kg. The children were weighed without shoes and wearing light clothing. All measurements were taken twice and repeated a third time if the first two measurements differed by more than 0.5 cm for BH or 0.5 kg for BW. Body mass index (BMI) was calculated as BW in kilograms divided by BH in meters squared. These measurements compared to the *2000 Centers for Disease Control and Prevention* (CDC) growth chart of BMI for children aged 2 to 20 years.[2] Subjects with BMI $\geq$ 95th percentile were classified as obese; those with BMI between 5t^{h} percentile and < 85th percentile were classified as normoweight children.

Data were processed and analyzed using *SPSS version 17.0* software, and presented in text and tables. The correlation between obesity and functional constipation was analyzed using Chi-square test. A P value of < 0.05 was considered to be statistically significant, with 95% confidence interval (95% CI).

Results

From a total of 200 students at the school, 45 children's parents refused to provide informed consent, leaving 155 children who underwent body weight and height measurements. Of these, 5 children were excluded because they were overweight (BMI between the 85th and 95th percentile). Hence, 150 subjects were either normal weight or obese. We divided subjects into two groups, with and without functional constipation. Subject's characteristics are shown in **Table 1**.

We assessed for a relationship between sex and functional constipation in our study. Chi-square test revealed no significant relationship between sex and functional constipation (P>0.05) (**Table 2**). However, Chi-square test revealed a significant relationship between obesity and functional constipation (P=0.0001) (**Table 3**).

Table 1. Subjects' characteristics

Variables	Functional constipation			
	Yes (n= 49)		No (n= 101)	
	Mean (SD)	95%CI	Mean (SD)	95%CI
Age, years	14.7 (1.04)	14.1 to 14.7	14.2 (1.00)	14.0 to 14.4
Body weight, kg	53.8 (15.10)	49.4 to 58.1	44.1(10.20)	42 to 46.1
Body height, cm	144.3 (7.20)	142 to 147.2	148.8 (7.50)	146.7 to 151
BMI, kg/m^2	23.5 (6.30)	21.7 to 25.3	20.7 (4.20)	19.8 to 21.6

Table 2. The relationship between sex and functional constipation

Variables	Functional constipation		P value
	Yes (n=49)	No (n=101)	
Male, n(%)	22 (35)	41 (65)	0.616*
Female, n(%)	27 (31)	60 (69)	

*Chi-square test

a risk factor for functional constipation.[9] Also in contrast to our results, a US study found that girls had 3 times higher risk of functional constipation than boys.[10] However, another US study concluded that gender was not a risk factor for functional constipation in children.[11]

Table 3. The correlation between obesity and functional constipation

Variables	Functional constipation		Prevalence ratio (95%CI)	P value
	Yes (n= 49)	No (n= 101)		
Obese, n(%)	18 (58)	13 (42)	4 (1.72 to 8.94)	0.001*
Normoweight, n(%)	31 (26)	88 (74)		

*Chi-square test

Discussion

In our study, the prevalence of functional constipation was 32.6%. Subjects with functional constipation had a mean age of 14.7 years. The prevalence of obese children with constipation was 58%. The prevalence of functional constipation worldwide was reported to range from 0.7 to 29.6%.[5] Loening-Baucke found that 22.6% of 482 children had functional constipation, and ranged in age from 4 to 17 years. Children under 1 year of age were excluded because their may be a greater likelihood of organic causes in children this young.[5]

In subjects with functional constipation, mean body weight and mean body height were 53.8 kg and 144.3 cm, respectively. The mean BMIs in children with and without constipation were 23.5 kg/m^2 (above 75th percentile) and 20.7 kg/m^2 (below 50th percentile), respectively. These results showed that children with functional constipation had higher BMI. Although BMI depends on race and gender, it remains the best tool to assess percentage of fat and the association between body weight and body height.[6,7] *The American Academy of Pediatrics* (AAP) recommends BMI monitoring to prevent obesity in children and adolescents.[8]

We found no significant association between biological sex and functional constipation. On the contrary, using the *Constipation Risk Assessment Scale*, a score of 2 for girls indicated that being female was

In our study, we found a significant correlation between obesity and functional constipation, with prevalence ratio of 4, indicating that obese children had four times higher risk of functional contipation compared to normoweight children. This result was similar to that of a retrospective study in the US in 2006 that showed 41% of obese children had functional constipation.[12] Furthermore, another study in 2004 explained that hormonal changes or hyperglycemia may have important roles in functional contipation on obese children.[4]

To assess all risk factors for functional constipation, univariate and multivariate analyses are needed. We used only Chi-square test in this cross-sectional study. As such, our preliminary data can be used as a basis for further study to evaluate other risk factors for functional constipation. Another limitation of this study was not using random sampling to choose subjects, hence, there may have been some bias.

In conclusion, obese children have four times higher risk of functional contipation than normoweight children. The prevalence for functional constipation in obese children in our study was 58%. We also observed no association between gender and functional constipation in children. Additional studies are required to identify other risk factors, such as eating patterns and binge eating, in order to improve our understanding of the mechanisms involved in functional constipation.

Conflict of Interest

None declared.

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Original Article

The addition of omeprazole to ondansetron for treating chemotherapy-induced nausea and vomiting in pediatric cancer patients

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Abstract

Background Chemotherapy-induced nausea and vomiting are some of the most disturbing side effects in pediatric cancer patients. The standard recommendation is the use of 5-hydroxytryptamine 3 receptor antagonist, such as ondansetron, to treat these symptoms. Despite this treatment, more than 50% of patients still experience nausea and vomiting.

Objective To evaluate the effect of the addition of omeprazole to ondansetron in the treatment of chemotherapy-induced nausea and vomiting.

Methods A double-blind, randomized, controlled trial was conducted at Haji Adam Malik Hospital, Medan, North Sumatera, from March to May 2016. Subjects were children aged 1 to 18 years, diagnosed with cancer, and who received intravenous chemotherapy. Patients were randomized to receive either a single dose of ondansetron (0.5 mg/kg) plus placebo or ondansetron (0.5 mg/kg) plus omeprazole (0.5 mg/kg). The severity of nausea and vomiting were measured using the Rhodes index of nausea, vomiting, and retching during the 24 hours after initiation of emetogenic chemotherapy. The primary outcome of efficacy was the proportion of patients who achieved complete response (lack of nausea/vomiting). Statistical analysis was performed by Chi-square and Fischer's exact tests.

Results Seventy eligible pediatric patients were randomized into two groups: 32 subjects in the ondansetron + placebo group and 38 others in the ondansetron + omeprazole group. The therapy failed in 50% (16/32) of the ondansetron + placebo group and 18.4% (7/38) of the ondansetron + omeprazole group. There was a significant difference in the clinical response between groups (P=0.01).

Conclusion The addition of omeprazole to ondansetron for the treatment of chemotherapy-induced nausea and vomiting is more effective than administration of ondansetron alone. **[Paediatr Indones. 2018;58:42-7 ; doi: http://dx.doi.org/10.14238/pi58.1.2018.42-7]**.

Keywords: *omeprazole; ondansetron; chemotherapy; vomiting; pediatric cancer*

Cancer is the second most common cause of death in children aged less than 15 years.[1,2] Cancer treatment generally consists of surgery, radiotherapy, and/or chemotherapy.[1] Chemotherapy is remains the first choice for cancer treatment in children.[1,2] Treatment with chemotherapy can cause side effects, the most common being nausea and vomiting.[3] Chemotherapy-induced nausea and vomiting (CINV) causes stress, dehydration, electrolyte disturbance, malnutrition, and anorexia, often resulting in patients refusing treatment at the next chemotherapy cycle.[4]

Serotonin receptor antagonists (ondansetron) are commonly used in the management of CINV in

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children.[5-9] Study on the use of antiemetics in children is still scarce, and more than 50% of patients continue to have vomiting, despite taking 5-hydroxytryptamine 3 receptor antagonists.[5,7,10] In general, chemotherapy causes nausea and vomiting through direct stimulation of the vomiting center, or indirectly through the chemoreceptor trigger zone (CTZ) and the peripheral vomiting center of the gastrointestinal tract.[11] Chemotherapy also results in damage to the gastrointestinal mucus,[12] increased gastrin hormone secretion,[13] delayed gastric emptying causing gastric distension,[14] and stress (psychological),[15-18] ultimately leading to an increase in gastric acid.[13-14] Proton pump inhibitors (such as omeprazole) function to reduce gastric acid[19] in dyspepsia syndrome, so we aimed to assess its effect in children with cancer who received chemotherapy.

Methods

A double-blind, randomized, controlled trial was conducted at Haji Adam Malik Hospital, Medan, North Sumatera, from March to May 2016. Subjects were children aged 1 to 18 years, diagnosed with cancer, who received intravenous chemotherapy, and with moderate to severe emetogenic risks. The exclusion criteria were patients with malignancy of the gastrointestinal tract, nausea or vomiting in the 24 hours before chemotherapy, other known causes of nausea or vomiting, or severe comorbidities such as malnutrition, encephalitis, meningitis, sepsis, bronchopneumonia, pulmonary tuberculosis, neutropenia, severe anemia, or severe hemorrhage. All patients were hospitalized during chemotherapy administration.

The minimum required sample size was calculated by using the sample formula for hypothesis testing of two independent proportions. The proportion of complete control of standard drugs (ondansetron) was 50%,5,8 and the difference in proportion of complete control between groups was expected to be 30%. Data on subjects' age, sex, type of cancer, and emetogenic levels of chemotherapy were collected along with demographic information as well as severity of nausea and vomiting. Data analysis was done with using the statistical package for social science (*SPSS), version 19.0* and the results presented in tables. This study was approved by the Health Research Ethics Committee at the University of Sumatera Utara Medical School.

All children who fulfilled the inclusion criteria were enrolled in this study. Subjects were divided into two groups by simple randomization. Group I received intravenous 0.5 mg/kg ondansetron and 0.9% NaCl 30 minutes before chemotherapy. Group II was given 0.5 mg/kg ondansetron 30 minutes before chemotherapy, and 0.5 mg/kg omeprazole (administered intravenously) shortly before chemotherapy. The treatments were carried out in a disguised manner in which the 0.9% NaCl and omeprazole (40 mg dry powder) were placed in new vials (labeled A for 0.9% NaCl and B for diluted omeprazole) with 8 mL (1 mL solution in vial B containing 5 mg omeprazole) each given at a dose of 0.1 mL/kg. The severity of nausea and vomiting was measured by the *Rhodes* index of nausea vomiting and retching (RINVR) during the 24 hours after initiation of emetogenic chemotherapy.20 We tested the validity RINVR by Pearson's correlation test and obtained Cronbach's coefficient alpha of 0.97. We interviewed children and parents to obtain demographic data. Nausea and vomiting indices were documented by a research assistant during chemotherapy, and by parents for 24 hours after chemotherapy.

Analyses of nausea and vomiting were done separately for the 24 hours after initiation of emetogenic chemotherapy. Severity of nausea vomiting based on 0-32 score range where no nausea vomiting: 0, mild: 1-8, moderate: 9-16, severe: 17-24, very heavy: 24-32. Clinical antiemetic response is divided into 3 groups: complete control (no nausea, vomiting), partial (mild and moderate), and failure (severe and very severe). In this study, the clinical response of the drug was divided into 2 groups: successful (complete control) and failed therapy (partial and failed control). The X test and Fischer's exact test were used to compare the difference in efficacy of the two antiemetic treatments. A P value of < 0.05 was considered to be statistically significant.

Results

Seventy eligible pediatric patients were randomized by simple randomization: 32 subjects in the ondansetron + placebo group and 38 in the ondansetron + omeprazole group. **Table 1** shows the baseline

characteristics of subjects by group, including sex, mean age, type of cancer, and emetogenic levels of chemotherapy. Both groups had more boys than girls. The most common types of cancer were leukemia (62.5% *vs.* 52.6%). The emetogenic levels of chemotherapy were mild 3/32 *vs.* 2/38, moderate 23/32 *vs.* 29/38 and high 6/32 *vs.* 7/38. Subjects' mean ages were 7.0 (SD 4.14) years in the ondansentron + placebo group, and 7.7 (SD 4.80) years in the ondansentron + omeprazole group.

Table 1. Demographic characteristics of subjects

Characteristics	Ondansetron + placebo (n = 32)	Ondansetron + omeprazole (n = 38)
Mean age (SD), years	7 (4.14)	7.7 (4.80)
Sex, n		
Male	20	22
Female	12	16
Malignancy type, n		
ALL	16	19
AML	3	1
CML	1	0
HL	2	4
NHL	2	3
Retinoblastoma	7	6
Rhabdomyosarcoma	1	1
Sarcoma	0	1
Teratoma	0	2
Testicular tumor	0	0
Chemotheraphy agent, n		
Carboplatin	7	3
Cysplatin	0	3
Cytarabin	2	0
Cyclophosphamide	6	7
Doxorubicin	13	23
Danurubicin	1	1
Vincristine	3	2
Emetogenic level, n		
Mild	3	2
Moderate	23	29
Severe	6	7

ALL: acute lymphoblastic leukemia, AML: acute myeloblastic leukemia, CML: chronic myeloblastic leukemia, HL: Hodgkin's lymphoma, NHL: non-Hodgkin's lymphoma

Table 2 shows the clinical response to the drugs in both groups. The treatment failed in 16/32 subjects in the ondansetron + placebo group, and 7/38 in the ondansetron + omeprazole group. The addition of omeprazole to ondansetron in managing CINV in pediatric cancer patients was more effective than ondansetron administration alone (RR 1.6; 95%CI 0.18 to 0.42; P=0.01). Kolmogorov-Smirnov test showed that the indices of nausea and vomiting were normally distributed.

Table 2. Clinical response to medication

Intervention	Clinical response		Total	P value
	Complete response	Failed response		
Ondansetron + placebo	16	16	32	0.01*
Ondansetron + omeprazole	31	7	38	
Total	47 (67.1)	23 (32.9)	70	

*Chi-square test

Discussion

The results of this study showed that the addition of omeprazole to ondansetron in the management of nausea and vomiting due to chemotherapy was more effective than (or superior to) ondansetron alone.

The gastrointestinal tract is involved in the mechanism of CINV through impulses carried to the peripheral vomiting center through the vagus and sympathetic nerves.[11] This system contributes to autonomic sensations such as gastric distension, gastric acid, anxiety, depression, and pain.[17,21] Increased autonomic activity against stress triggers an increase in gastric acid, in which the vagus nerve stimulates parietal cells directly or through gastrin antral effects by releasing gastric-releasing peptide (GRP), acetylcholine bound to M3 muscarinic receptors, and histamine.[22] Serotonin release from enterochromaffin cells due to chemotherapy leads to stimulation of peripheral vomiting centers and stomach muscle dysmotility.[11] Increased gastric acid secretion directly stimulates the vomiting center.[21-22]

Riezzo *et al.* showed that abnormalities in gastric motor activity result from loss of regular activity of slow waves.[13] Also, Nelson *et al.* reported that gastric dysrhythmias were associated with anterior hypomotility and delayed gastric emptying times, with symptoms such as nausea and vomiting.14 These findings suggest that changes in gastric electrical activity are associated with symptoms of dyspepsia, rather than symptoms of vomiting. The conclusion to

the studies was that chemotherapy causes symptoms of nausea and vomiting related to a dyspepsia syndrome.[13,23] The distension that occurs in the stomach stimulates tense receptors that eventually stimulate gastric acid secretion by parietal cells.[22,24]

During chemotherapy, cell injury in the gastrointestinal tract causes release of several inflammatory factors including cyclooxygenase-2 (COX-2), interleukin-6 (IL-6), and nuclear factor kappa-B (NF-kB).[21,25-26] The end result of this pathway is tissue damage and potential mucositis that continues along the gastrointestinal tract.[12] Inflammation and cell injury are thought to be heavily involved in delayed CINV.[21]

A randomized, cross-over, and double-blinded study in India by Sontakke et al. treated CINV with ginger and obtained complete control of 62%.[27] Pillai *et al.* reported that ginger powder was effective in reducing severity of acute and delayed CINV in patients receiving highly emetogenic chemotherapy. They concluded that ginger improved gastric motility.[28] The secretion of gastric acid decreased such that stimulation of the vomiting center was reduced.[22,24] This is consistent with the foundation of our research theory.

Deghani et al. reported that the omeprazole dose of 1 mg/kg/day in children was superior to ranitidine, famotidine, and cimetidane in reducing dyspepsia symptoms of nausea (86.2%), vomiting (80.8%), and flatulence (79.5%).19 In addition, Sartori *et al.* reported that 20 mg of omeprazole reduced gastric acid production by 97% and maintained gastric pH for 18 to 20 hours. They also suggested that gastric mucosal injury due to chemotherapy can be prevented with omeprazole (P=0.001).[29] In our study, a dose of 0.5 mg/kg omeprazole was administered intravenously before chemotherapy. We chose this dose in consideration of the severity of stress conditions from cancer, while still in the drug dose range.

Standard treatment of CINV is based on the emetogenic potential of the chemotherapy used.[5,6,10] Holdsworth *et al.* found moderate emetogenic risk in 63.4% of girls and 64.1% of boys, and high risk in 36.6% of girls and 35.9% boys.[8] Similarly, Hilarius *et al.* found moderate emetogenic risk of 63% and high risk of 37% in a community-based hospital study.[30] Female and younger patients are more likely to experience CINV than male and older patients.[5,10] In our study, we found no significant differences in emetogenicity between the ondansetron + placebo and ondansetron + omeprazole groups: low risk 9.4% *vs.* 5.3%, respectively, moderate risk 71.9% *vs.* 76.3%, respectively, and high risk 18.8% *vs.* 18.4%, respectively (P=0.795). In other studies, children undergoing moderate and high emetic risk chemotherapy were recommended to receive a serotonin receptor antagonist (ondansetron) in combination with an NK-1 receptor antagonist (aprepitant) and dexamethasone.[5,6,10] To date, no randomized trial of aprepitant has been performed in children.[5] Our study was a pilot study to assess the effectiveness of the addition of omeprazole to ondansetron for treating CINV in pediatric cancer patients.

A double-blind, randomized study by Siddique *et al.* reported that complete and partial responses to CINV in ondansetron administration alone were 70% and 30%, respectively, for the acute type, and 43% and 50%, respectively, for the delayed type.[31] Jaing *et al.* reported that administration of 0.15 mg/kg ondansetron gave a complete response of 45.5%.[7] In addition, Holdsworth *et al.* reported that complete response to ondansetron (0.45 mg/kg IV) was seen in 65.5% of patients.[8] Also, Kurucu *et al.* noted that 5 mg/m^2 intravenous ondansetron gave a complete response for the acute type in 55% of patients.[9] These findings suggest that ondansetron is not optimal in the management of CINV, the experience of nausea and vomiting is highly subjective,[32] and appropriate dosing strategies for children and a combination of drugs are needed.[33]

In our study, successful therapy (complete response) with 0.5 mg/kg ondansetron was 50% (16/32). However, the addition of 0.5 mg/kg body weight omeprazole to ondansetron yielded an 81.6% (31/38) therapy success (RR 1.6; 95%CI 0.18 to 0.42; P=0.01). The addition of omeprazole to ondansetron was very effective in the management of nausea and vomiting due to chemotherapy. The strength of this study was its experimental design: randomized, controlled, and double-blinded. The limitation of this study was the small sample size. As this was a pilot study, further investigation is needed to determine the efficacy of other proton pump inhibitors in pediatric cancer as an addition to standard treatment for CINV with a larger study sample size.

In conclusion, the addition of omeprazole to ondansetron for treatment of chemotherapy-induced nausea and vomiting is more effective than administration of ondansetron alone.

Conflict of Interest

None declared.

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Original Article

Prescribing antibiotics to pediatric dengue patients: increasing risk of bacterial resistance

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Abstract

Background Use of antibiotics to treat self-limiting viral infections like dengue fever (DF) without any co-morbid conditions in pediatric patients is common practice in India, and a major contribution of the inappropriate use of antibiotics in the country.

Objective To provide an analysis of diagnosis, grading, and prescribing of antibiotics in pediatric inpatients with DF in a tertiary care teaching hospital in India.

Methods Data from case sheets of all pediatric inpatients (n=370) diagnosed with DF without co-morbid conditions were collected with regards to diagnosis, grading, presence, and appropriateness of antibiotic usage according to the *2009 WHO Guidelines*, the *National Vector Borne Disease Control Program (NVBDCP) of India Guidelines*, and the *Hospital Infection Society (HIS) Guidelines*.

Results Platelet count determination (50% of the cases) was the major diagnostic method for dengue. Inappropriate grading of DF was seen in 20% of patients. Almost 75% of the 370 dengue cases were prescribed antibiotics for the expressed purpose of avoiding hospital-acquired infections. A single antibiotic was given in 225 prescriptions (60.81%), 2 antibiotics in 33 (8.91 %) cases, and 3 antibiotics in 9 (2.43%) cases.

Conclusions Prescribing one or more antibiotics to treat self-limiting viral infections is considered as inappropriate and may lead to the development of multidrug resistance. Furthermore, excess use of antibiotics in infancy may induce imbalances in gut and microbiota, called dysbiosis, and increases the probability of occurrence of diseases such as obesity, diabetes, and asthma in later life. These findings can inform the development of antibiotic stewardship in the treatment of dengue. **[Paediatr Indones. 2018;58:53-8; doi: http://dx.doi.org/10.14238/pi58.1.2018.53-8]**.

Keywords: *antibiotics; bacterial resistance; dengue; inappropriate use*

In the field of medicine, the battle between bacteria and mankind can be explained in three phases: the pre-antibiotic era, the antibiotic era, and the post-antibiotic era. The period before the introduction of sulfa drugs and penicillin is considered to be the 'pre-antibiotic era'. Bacteria dominated mankind and bacterial infections were the leading cause of death.[1] Discovery of penicillin by Sir Alexander Fleming in 1928 laid the foundation and hope of controlling bacterial infections. Since that time, the 'antibiotic era' has seen the discovery of many antibiotics, which has transformed modern medicine and saved millions of lives.[2] These discoveries gave hope that mankind would rein over bacterial infections forever. However, the foremost driving force for discovering newer antibiotics was the development of resistance to the existing antibiotics. Eventually, the antibiotic pipeline began to dry up, because the pharmaceutical industry considers

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investment in antibiotic study to be unprofitable, fearing a possible lack of returns. This approach of the pharmaceutical industry is due to the short period of antibiotic usage compared to other drugs used to treat metabolic, cardiovascular, and central nervous system disorders. Hence, bacterial resistance to antibiotics is inevitable, as newer antibiotics may be outdated in a short span of time.[2]

In response to antibiotics, bacteria change in ways that reduce or eliminate drug effectiveness. These changes are due to the evolution of antibiotic resistance genes by spontaneous mutations and furthered by natural selection of resistant strains over sensitive ones.[3] Once a strain develops resistance, the once-effective antibiotic will no longer inhibit the bacteria. If this continues, mankind will eventually face a cataclysmic condition. In view of this, the US *Centers for Disease Control and Prevention* (CDC) declared in 2013 that the human race is now in the 'post-antibiotic era'. Moreover, the *World Health Organization* (WHO) warned of a dreadful antibiotic resistance crisis.[2] This situation is comparable to the pre-antibiotic era, in that irrational use of antibiotics has led to the emergence of resistant strains and infections which are not yet recognized and the re-emergence of virulent forms of previous infections.[1] Resistance development is an evolutionary process, with unpredictable times. Resistance requires constant exposure of bacteria to the antibiotic. Hence, the rational or irrational use of antibiotics drives the evolution of bacterial resistance. As such, the greater the exposure of bacteria to antibiotics, the faster the evolution of resistance.[3]

Dengue and chikungunya are the third and fourth most common monsoon diseases in India. They are mosquito-borne (*Aedes aegypti*) viral diseases associated with urban environments. Dengue manifests as sudden onset of fever and severe headache; it occasionally produces shock and hemorrhage leading to death. Chikungunya is characterized by pain usually lasting 3-7 days and, in some cases, results in persistent arthritis. These diseases share common symptoms of a typical bacterial infection, and mislead health workers to use antibiotics unnecessarily. In addition, illicit prescribing of antibiotics by medical practitioners is increasing the condition of resistance, due to improper diagnosis, lack of understanding of the potential dangers of inappropriate use, costs, and outcomes of therapy.[4] During our hospital visit as per the curriculum of Pharm. D. course in the pediatric ward, we observed the use of antibiotics to treat diseases which are generally self-limiting, to avoid hospital-acquired infections in Sri Venkateswara Ramnarayana Ruia Government General Hospital (SVRRGGH), Tirupathi. Hence, we aimed to evaluate the extent of inappropriate prescribing of antibiotics in treating dengue fever (DF) in pediatric patients.

Methods

This cross-sectional, observational study was carried out for 6 months (July to December 2016) in the inpatient Department of Pediatrics, SVRRGGH, Tirupathi, India. The minimum required sample size (n=370) used was based on the pilot study that was performed in the early weeks of July 2016 on 50 patients.

The study was approved by the Institutional Review Board, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupathi, India. All patients admitted to the pediatric inpatient ward of SVRRGGH with dengue fever during the study period were included in the study, except for those with other infections and co-morbid conditions.

A specially designed pro forma was used for collecting data, including patient demographics, past medical history, family and surgical history, traveling and transfusion history, signs and symptoms, diagnosis, and medications presently prescribed for each patient. The data were obtained from patient case profiles after obtaining parental informed consent. All prescriptions were analyzed for the appropriate diagnosis, grading, and presence of antibiotics, according to the *2009 WHO Guidelines*[5] and *National Vector Borne Disease Control Programme (NVBDCP) Guidelines of India*.[6] In addition, the presence of appropriate antibiotics for hospital-acquired infection was also assessed based on *Hospital Infection Society (HIS) Guidelines of India*.[7-9]

Results

In this study, we observed more DF cases (**Table 1**) in children between the age group 5-10 years (52.97%), followed by 0-4 years (37.29%) and 11-15 years (9.23%).

Out of 370 cases, 181 (48.91%) cases were diagnosed based on platelet count, followed by NS1 antigen (36.75%), IgM (12.7%) and IgG (1.62%) antibodies, while only 4.4% of cases were diagnosed solely based on symptoms (**Table 2**).

In this study, 315 (85.13%) cases were appropriately graded and 72 cases (19.45%) were inappropriately graded (**Table 3**). Of the 315 appropriately-graded cases, mild dengue was observed in 132 cases (41.9%), moderate dengue in 176 cases (55.87%), and severe dengue in 7 cases (2.22%). Among the moderate and severe cases, DHF prevalence was 49.45% (183 subjects) in which DHF1 contributed to the highest percentage (48.63%; 89 cases), followed by DHF 2 (47.54%), and DHF 3 (3.82%).

Of 370 confirmed dengue cases, 267 (74.6%) cases were prescribed antibiotics. A single antibiotic was prescribed to 225 cases (60.81% of all cases), 2 antibiotics to 33 (8.91%) cases, and 3 antibiotics to 9 (2.43%) cases, at a time (**Figure 1**). Triple therapy antibiotics included cefotaxime in all prescriptions with cefixime, azithromycin, amoxyclav, doxycycline, and ceftriaxone in different combinations. Antibiotics given as dual therapy were ceftriaxone with doxycycline, cefotaxime, or amoxyclav, and cefotaxime with doxycycline, cefixime, or metronidazole.

Among the antibiotics prescribed, cefotaxime and ceftriaxone were most commonly used (97 cases; 30.50% for each drug), followed by doxycycline (17.92%), amoxyclav (10.06%), cefixime (3.77%), amikacin (2.83%), azithromycin (2.51%), as well as ciprofloxacin and metronidazole (0.94%) (**Table 4**).

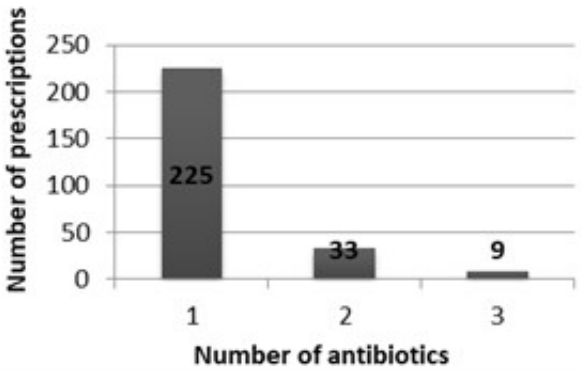

Figure 1. Distribution of antibiotics

Table 1. Age distribution of dengue fever patients

Age group	No. of cases (N=370)
0-4 years	138
5-10 years	196
11-15 years	36

Table 2. Diagnostic parameters used to diagnose dengue fever

Parameters	No. of cases (N=370)
PLT, n (%)	181 (48.91)
NS1AG, n (%)	136 (36.75)
IgM, n (%)	47 (12.7)
IgG, n (%)	6 (1.62)

Table 3. Appropriateness in grading dengue fever

Grading	No. of cases (N=370)
Inappropriate, n (%)	72 (19.45)
Appropriate, n (%)	315 (85.13)

Table 4. Antibiotic prescribing pattern

Antibiotic	No. of prescription (N=267)
Cefotaxime, n(%)	96 (35.9)
Ceftriaxone, n(%)	96 (35.9)
Doxycycline, n(%)	37 (13.8)
Amoxyclav, n(%)	23 (8.6)
Cefixime, n(%)	7 (2.6)
Amikacin, n(%)	4 (1.5)
Azithromycin, n(%)	2 (0.75)
Ciprofloxacin, n(%)	1 (0.38)
Metronidazole, n(%)	1 (0.38)

Discussion

In this study we found that dengue was more prevalent in the age group of 5-10 years. This may

be because of children playing in unsanitary places or field areas where there is risk of being bitten by mosquitoes. There is no existing scientific reason for high prevalence of dengue in children, but the rate of mortality in pediatrics is high due to secondary infections, developing immunity, and exposure to virulent strains, while most children remain asymptomatic.[10]

The most common monsoon diseases in India are malaria, diarrhea, dengue, chikungunya, typhoid, viral fevers, and cholera. They all share some common symptoms and present challenges for the physician. Antibiotics are of no use in management of dengue and chikungunya, as they are viral, infections. According to the *WHO Clinical Guidelines*[5] and *the 2014 NVBDCP Guidelines*,[6] no drug of choice for dengue exists, as drugs that directly act against dengue virus are still in the pipeline.[11] Hence, therapy is solely based on the management of symptoms. Accurate diagnosis of dengue can be confirmed based on serum positivity for NS1 antigen, IgM, and IgG antibodies. The NS1 antigen can be determined by ELISA, which is highly sensitive, specific,[12] and helpful in the early diagnosis of acute infection. In our study, we observed that platelet count determination had been used in most (50%) of the cases to diagnose dengue infection. This approach is not appropriate because thrombocytopenia occurs due to destruction of platelets, mediated by antiplatelet-antibodies observed from the 3rd day to the 7th day of illness. Moreover, platelet count may also fall in other infections like HIV, HHV-6, ehrlichiosis, Rickettisia, malaria, hepatitis-C, cytomegalovirus, Epstein-Barr syndrome, *Helicobacter pylori*, and *E. coli.* Hence, platelet count is not an ideal diagnostic parameter to diagnose dengue fever. However, it can be considered for prognosis of the disease. These implementations can make the physician clear and confident regarding usage of antibiotics and further allows him to follow strict regimen recommended by *WHO* and *HIS of India* and thus reduce the need for antibiotic use and cost burden on the patient.

Once the dengue diagnosis has been confirmed, grading plays a major role in the success of the treatment. Inappropriate grading may result in failure of therapy, and the need for advanced assessment of complications. As manifestations of DF vary by grades, different treatment approaches are required, as mentioned in the *WHO Guidelines*. Mild dengue does not require therapy with IV fluids, while moderate and severe dengue requires IV fluid maintenance. Moderate dengue can be managed with IV fluids at a rate of 2 to 3 mL/kg/hr, with reassessment of CBC to increase the rate up to 5 to 10 mL/kg/hr for 2 hours. In severe dengue, hemodynamic status of the patient requires assessment, for the basis of the physician to decide between a bolus of 20 mL/kg in 15 minutes or maintenance with IV fluids at a rate of 5 to 10 mL/kg/hr over 1 hour. Otherwise, DF may be fatal after entering into the phases like shock and coma.[5,6]

In our observation, 75% of the cases were prescribed with antibiotics, under the guise of avoiding hospital-acquired infections. However, antibiotics are not necessary to treat dengue, according to the guidelines. Empiric antibiotics are being prescribed for suspected infections to avoid hospital-acquired infections. However, this empiric use of antibiotics for treating nosocomial infections should be according to the HIS guidelines. In addition, antibiotics like amikacin, doxycycline, and amoxyclav should not be used without performing susceptibility tests, while ceftriaxone and cefixime cannot be used prior to performing hypersensitivity tests.[13-16] Azithromycin, ceftriaxone, and metronidazole can only be used for the prophylaxis of endocarditis and sexually transmitted diseases (STDs).[17,18] In addition, usage of amoxyclav in dengue can further increase the risk of bleeding.[14]

The prescribing of antibiotics is an art requiring skill in using the appropriate antibiotic for the particular infection. Inappropriate usage of more than one antibiotic increases the chances of developing multidrug resistance, which is a serious issue to be considered. Though there are many policies describing the appropriate indications for use of antibiotics, these drugs are often used inappropriately. Furthermore, if this scenario continues, the list of organisms developing resistance will increase. More powerful and costlier antibiotics must be prescribed for simple infections, which may have many adverse effects and can present an economic burden to patients.

In conclusion, prescribing one or more antibiotics to treat self-limiting viral infections is considered as inappropriate, and leads to the development of multidrug resistance. Furthermore, excess use of antibiotics in infancy may induce imbalances in gut and microbiota, called dysbiosis, and increase the probability of diseases such as obesity, diabetes, and asthma in later life.[19] These findings can be used to improve antibiotic stewardship in the treatment of dengue.

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Conflict of Interest

None declared.

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Original Article

Infant feeding practice on growth velocity in 4 to 6-month-olds

Joko Kurniawan, Alifah Anggraini, Madarina Julia

Abstract

Background In developing countries, 5-10% of infants suffer from failure to thrive. Adequate feeding is the most crucial factor for optimal growth in early life.

Objective To assess the differences in growth velocity at 4 to 6 months of age, based on the infant feeding practices.

Methods This cross-sectional study involving 4 to 6 month-old babies from 6 public health centres in Yogyakarta was performed from August to November 2016. Data on body weight, and growth velocity as they related to weight at birth were collected. Subjects were divided into groups according to their feeding practices.

Results Of 173 subjects, 130 (75%) infants were exclusively breastfed, 19 infants (11%) were given breast milk and formula, 14 (8%) infants were given breast milk and complementary food (8%), and 10 (6%) infants were given formula and complementary food. The mean growth velocity z-scores by group were as follows: exclusively breastfed 0.04 (SD 1.15) (95%CI -0.16 to 0.24), breast milk and formula -0.61 (SD 0.84) (95%CI -1.01 to -0.21), breast milk and complementary food -0.69 (SD 1.14) (95%CI -1.35 to -0.04), formula and complementary food 0.23 (SD 1.50) (95%CI: -0.84 to 1.31). The mean difference in growth velocity between the exclusively breastfed vs. breast milk and formula groups was 0.65 (SD 0.28) (95%CI: 0.10 to 1.20; P=0.02); vs. breast milk and complementary food was 0.73 (SD 0.32) (95%CI: 0.10 to 1.37; P=0.02); and vs. formula and complementary food was -0.19 (SD 0.37) (95%CI: -0.93 to 0.55; P=0.61).

Conclusion Exclusively breastfed have the most optimal growth velocity compared to infants who experience other feeding practices. **[Paediatr Indones. 2018;58:36-41 ; doi: http://dx.doi.org/10.14238/pi58.1.2018.36-41]**.

Keywords: *growth velocity; feeding practice; infants*

Failure to thrive in infants is found in approximately 5-10 % children in the developing countries.[1] Early detection of failure to thrive in the first year of life is important to prevent negative impacts on children's development. One of the most important factors in growth is sufficient nutrient intake to optimize the growth process.[2] In children aged 0-1 years, especially in the first 6 months, breast milk plays an important role in infant feeding. Kramer *et al.* concluded that exclusive breastfeeding could prevent the incidence of failure to thrive in the first year of life.[3] However, another study showed a slower growth velocity in breastfed babies.[4]

In Indonesia, exclusive breastfeeding is recommended for infants aged 0-6 months. Several

This study was presented at the *Kongres Nasional Ilmu Kesehatan Anak (KONIKA) XVII* (The 17th National Congress of Child Health), Yogyakarta, Indonesia, August 8-11, 2017.

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previous studies on breastfeeding and growth had varying results.[3-5] The aim of this study was to assess infant growth velocity associated with pattern of feeding, i.e. either exclusive breastfeeding, a combination of breastfeeding and formula feeding, a combination of breastfeeding and complementary food, or formula feeding only.

Methods

A cross sectional study was performed from August to November 2016 in six public health centers (Pusat kesehatan masyarakat, Puskesmas). The six health centers were randomly selected from 18 health centers in Yogyakarta: Puskesmas Jetis, Gedongtengen, Danurejan I, Gondokusuman I, Mergangsan, and Umbulharjo I. The study was approved by the Medical and Health Research Ethics Committee, Universitas Gadjah Mada Medical School. Written informed consent was obtained from subjects' parents or legal guardians. There was no commercial support for the trial.

A total of 173 infants were consecutively selected to participate. Every Puskesmas contributed in proportion to the number of infants aged 0-6 months from that Puskesmas in 2014: 27 infants from Jetis, 20 from Gedongtengen, 11 from Danurejan I, 21 from Gondokusuman I, 46 from Mergangsan, and 48 from Umbulharjo I. The inclusion criteria were infants aged 4-6 months who were born in Yogyakarta, born full term with normal weight, had no history of serious illness or chronic disease (based on previous hospital care history), and lived in Yogyakarta. Patients were excluded if they had signs of major congenital anomalies.

Data on birth weights and feeding practices were obtained using questionnaires. Birth weight data were confirmed by the child's growth chart (*Kartu Menuju Sehat, KMS*). Data on food consumption were recalled by parents in the last 24 hours prior to visiting the Puskesmas. Body weight measurements were performed by trained health workers. The children were weighed on calibrated scale (with accuracy of 0.01 kg) in a supine position without clothes. Growth velocity was defined as the difference between the current body weight and the birth weight. The results were converted to *z*-scores using the *World Health Organization* (WHO) growth rate for age table.[1,6] Children whose rate of growth was between -2 and +2 standard deviation (SD) has normal weight gain. Failure to thrive was diagnosed in children whose rate of growth was less than -2 SD or the changes were less than 5th percentile. Growth was considered rapid when the growth rate was more than +2 SD. The growth velocities *z*-scores between feeding practice groups were compared using ANOVA, Fischer's least significant difference (LSD), and post hoc ANOVA tests.

Results

Of 173 infants from 6 Puskesmas who fulfilled the inclusion criteria, 101 (58.4%) were boys and 72 (41.6%) were girls. Most of the babies were delivered vaginally [133 (76.9%) vs. 40 (23.1%) caesarean section deliveries]. There were 99 (57.2%) infants aged 4 months, 54 (31.2%) infants aged 5 months, and 20 (11.6%) infants aged 6 months. Characteristics of the study subjects, stratified by age, are presented in **Table 1**.

Mean *z*-scores of body weight increment by feeding practice group are presented in **Table 2**. **Table 3** shows the weight increment in each type of feeding practice. Most babies had normal weight gain. One-way ANOVA revealed significant differences among the four groups (P=0.02). Post hoc test showed that the mean growth velocity *z*-score of the exclusively breastfed group [0.65 (SD 0.28)] was significantly higher than that of the breast milk and formula group (P=0.02), as well as that of the breast milk and complementary food (P=0.02). However, weight increment *z*-scores were not significantly different between the exclusively breastfed and the formula and complementary food groups (P=0.61) (**Table 4**).

Table 1. Characteristics of the study subjects stratified by age

Infants age stratification	Feeding practice			
	Exclusively breastfed (n=130)	Breast milk and formula (n=19)	Breast milk and complementary food (n=14)	Formula and complementary food (n=10)
Mode of delivery, n(%)				
Vaginal				
4 months	56 (82.4)	9 (13.2)	3 (4.4)	0
5 months	41 (89.1)	2 (4.3)	2 (4.3)	1 (2.2)
6 months	9 (47.4)	2 (10.5)	7 (36.8)	1 (5.3)
Total	106 (79.7)	13 (9.8)	12 (9.0)	2 (1.5)
Caesarean section				
4 months	20 (64.5)	3 (9.7)	1 (3.2)	7 (22.6)
5 months	4 (50.0)	3 (37.5)	0	1 (12.5)
6 months	0	0	1 (100.0)	0
Total	24 (60.0)	6 (15.0)	2 (5.0)	8 (20.0)
Level of maternal education, n(%)				
Senior high school or lower				
4 months	56 (77.8)	8 (11.1)	4 (5.6)	4 (5.6)
5 months	34 (82.9)	4 (9.8)	2 (4.9)	1 (2.4)
6 months	3 (27.3)	1 (9.1)	7 (63.6)	0
Total	93 (75.0)	13 (10.5)	13 (10.5)	5 (4.0)
College, graduate, or higher				
4 months	20 (74.1)	4 (14.8)	0	3 (11.1)
5 months	11 (84.6)	1 (7.7)	0	1 (7.7)
6 months	6 (66.7)	1 (11.1)	1 (11.1)	1 (11.1)
Total	37 (75.5)	6 (12.2)	1 (2.0)	5 (10.2)
Maternal occupation, n(%)				
Senior high school or lower				
4 months	51 (81.0)	7 (11.1)	2 (3.2)	3 (4.8)
5 months	33 (86.8)	3 (7.9)	1 (2.6)	1 (2.6)
6 months	4 (50.0)	0	4 (50.0)	0
Total	88 (80.7)	10 (9.2)	7 (6.4)	4 (3.7)
College, graduate, or higher				
4 months	25 (69.4)	5 (13.9)	2 (5.6)	4 (11.1)
5 months	12 (75.0)	2 (12.5)	1 (6.3)	1 (6.3)
6 months	5 (41.7)	2 (16.7)	4 (33.3)	1 (8.3)
Total	42 (65.6)	9 (14.1)	7 (10.9)	6 (9.4)

Table 2. Comparison of weight increments z-scores based on feeding practices

Group	Weight increment Z-scores		P value
	Mean (SD)	95%CI	
Exclusively breastfed	0.04 (1.15)	-0.16 to 0.24	0.02
Breast milk and formula	-0.61 (0.84)	-1.01 to -0.21	
Breast milk and complementary food (MPASI)	-0.69 (1.14)	-1.35 to -0.04	
Formula and complementary food	0.23 (1.50)	-0.84 to 1.31	

MPASI= makanan pendamping ASI = complementary food

Table 3. Weight gain comparison based on feeding practies

Feeding practices	Weight gain		
	Rapid, n(%)	Normal, n(%)	Failure to thrive, n(%)
Exclusively breastfed (n=130)	5 (3.8)	124 (95.4)	1 (0.8)
Breast milk and formula (n=19)	0	18 (94.7)	1 (5.3)
Breast milk and complementary food (MPASI) (n=14)	0	13 (92.9)	1 (7.1)
Formula and complementary food (n=10)	0	9 (90)	1 (10)

MPASI= makanan pendamping ASI = complementary food

Table 4. Post hoc comparison of weight increment z-score between feeding practice groups

Comparison between feeding practices		Mean of differences (SD)	95%CI	P value
Exclusive breastfed	Breast milk and formula	0.65 (0.28)	0.10 to 1.20	0.02
	Breast milk and complementary food (*MPASI*)	0.73 (0.32)	0.10 to 1.37	0.02
	Formula and complementary food	-0.19 (0.37)	-0.93 to 0.55	0.61
Breast milk and formula	Breast milk and complementary food (*MPASI*)	0.09 (0.40)	-0.71 to 0.88	0.83
	Formula and complementary food	-0.84 (0.45)	-1.72 to 0.04	0.06
Breast milk and complementary food (*MPASI*)	Formula and complementary food	-0.93 (0.47)	-1.86 to 0.01	0.05

MPASI= makanan pendamping ASI = complementary food

Discussion

This study showed that exclusive breastfeeding provided the most optimal growth velocity for infants aged 4-6 months compared to other feeding practices. This result was also supported by the post hoc analysis of the mean (SD) difference of the growth velocity Z-scores, despite the fact that all feeding groups had infants who failed to thrive. We suggest that optimal growth velocity is related to breast milk composition, as it contains the ideal nutrient for infants, especially in the first 4 months of life compared to other foods.

Not only important for growth, breast milk also increases infant immunity and intelligence, and may improve the emotional connection between mother and baby.[7,8] Breast milk contains several protective factors that increase infant immunity in the first 6 months. Lactoferin in breast milk binds iron which may inhibit bacterial growth, as bacteria require iron. Lysozyme is another protective factor that destroys bacterial cell walls. Breast milk also has lipoprotein lipase involved in the lypolysis of triglycerides in breast milk to produce monoglycerides and free fatty acids, linoleic acid as a precursor of prostaglandin and leucotrien.[7] Immunoglobulin in breast milk provide local protection of the gastrointestinal mucosa from pathogens.

The incidence of failure to thrive was found in every feeding practice group, including the exclusively breastfed group (**Table 3**). The number and frequency of feedings were not calculated in this study, so we could not assess infants' caloric intake.[9,10] Similar with the result of this study, previous studies found that breast milk can prevent the incidence of failure to thrive compared to other foods.[3, 11] The incidence of failure to thrive in children is influenced by many factors, such as: parental opinions regarding feeding practices,[12] prematurity,[13] the amount of energy and protein intake from breast milk and other foods,[5] feeding of variety of foods after 6 months of age,[14] maternal factor in food introduction in children under 1 year of age,[15] and socioeconomic status of the family.[16]

Growth velocity is the change in body weight by time (month) or gram/month compared to population according to age. The growth velocity can be measured from body weight, head circumference, and body length. Body weight is the main indicator, as it is useful in the short term. Head circumference has been used as the second indicator, while the body length is

useful for detection of stunting, because its usefulness over a longer period of time.[17] Changes of less than 5th percentile or less than -2 SD are interpreted as a failure to thrive. Changes in body weight can be seen in growth velocity or growth increment tables with intervals of 1-6 months.[11]

We noted that the highest growth velocity was not found in exclusively breastfed group, but in the formula fed group. This finding was consistent with previous studies which reported that formula feeding increased the risk of obesity in children.[17,18] Another study also noted that growth velocity in the exclusively breastfed group was slower than in other feeding practice groups.[4] An optimal growth velocity is expected to support the optimal growth and development in children.[2,19]

In conclusion, there is a significant difference in the mean growth velocity Z-scores of infants aged 4-6 months according to feeding practices. Exclusive breastfeeding provides the most optimal growth velocity (mean Z-score tend to 0) compared to other feeding practice groups.

The limitation of this study was that causal relationships between the variables could not be explained. Exclusive breastfeeding for 6 months remains the main recommendation according to the existing results.

Conflict of interest

None declared.

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Original Article

Food allergies in children: a comparison of parental reports and skin prick test results

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Abstract

Background Food allergy is common in children and its prevalence is generally on the rise. Imprecise parental reports about reactions to particular foods can lead to unnecessary restrictions. Since children have specific growth requirements, such nutritional restrictions may have disturbing effects on children's growth and development.

Objective To compare parental reports on food reactions to skin prick test results in their children.

Method Retrospective, cross sectional study using patient's medical record data during one-year study period. Data were analyzed manually and statistically, to assess the degree of agreement (Kappa's coefficient) and significance (P).

Results We collected data from 154 subjects aged 0-18 years. For every allergen assessed, parents reported more food reactions than positive skin prick test results. Allergy incidence were caused, in order, by cow's milk and chicken (25.3%), eggs (22.1%), chocolate (20.1%), fruits (14.3%), seafood (13%), and saltwater fish (1.9%). Kappa coefficient are all poor (<0.2) and P value are all >0.05 except for chicken (P=0.02).

Conclusion Most parents tend to overestimate which food cause reactions in their children, as reactions reported were not necessarily allergenic. Therefore, every patient experiencing allergy reactions should undergo skin prick testing to confirm the possibility of allergy. **[Paediatr Indones. 2018;58:59-65; doi: http://dx.doi.org/10.14238/pi58.1.2018.59-65]**.

Keywords: *food allergy; skin prick test*

Food allergy is an abnormal reaction towards certain food antigens to some individual.[1] Food allergy is common in children. Its manifestations range from skin to respiratory system. Foods with high nutritional value such as cow's milk, eggs, chicken, and seafood may cause these adverse reactions.[2] Since children have increased nutritional needs for proper growth and development, the imprecision of parental estimation about particular food causing reactions can lead to unnecessary restrictions of certain food. Such restrictions may disturb children's growth and development.

Diagnostic testing is needed for food allergy diagnosis since medical history and physical examination are not sufficient. Skin prick testing is recommended[3] because it is fast, inexpensive, reliable, widely available, and has been widely studied. Negative test results have been shown to effectively rule out Ig-E mediated allergies.[4] We aimed to determine whether parental reports corresponded to the skin prick test results in children with food reactions.

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Methods

We conducted a retrospective cross-sectional study at the Department of Pediatrics, Dr. Soetomo General Hospital, Surabaya. We reviewed medical histories of patients who visited the hospital from 1 January 2015 until 31 December 2015. There were 154 patients throughout the year. The inclusion criteria were patients aged 0-18 years old, had allergy symptoms, had parents who had reported possible food allergens, and who underwent a skin prick test in the Department of Child Health during 2015. Data were collected by total sampling.

Allergy symptoms had been collected through interviews with the parents and was written on the 'anamnesis' columns, completed with details of the symptoms. Possible food allergens were also been collected through interviews and was written on 'possible food allergens' column. Skin prick test results were performed with sterile lancets and commercial food allergens extract in the volar area of forearm, on columns which had been drawn. Seven allergens were tested: fruits, seafood, saltwater fish, cow's milk, chocolate, chicken, and egg. Control specimen used were normal saline (negative) and histamine (positive). Reactions were observed after 20 minutes. The wheal-and-flare reactions were measured with a ruler in millimeters (1 mm = 0.001 m). The vertical and horizontal diameters of the wheal-and-flare were added and divided by 2, resulting in mean diameter, which was recorded. Skin prick test results were considered to be positive if their diameter was >3mm than the negative control. The supervising pediatrician made the final diagnoses. Patient's age and sex were also collected from medical records.

The distribution pattern was shown in a descriptive table. We used *SPSS* software to determine the degree of agreement (Kappa's coefficient) and significance (P value). Results with P values <0.05 were considered to be statistically significant. This study was approved by the Ethics Committee of Dr. Soetomo General Hospital, Surabaya.

Results

One hundred fifty-four children were enrolled in this study. Subject's characteristics are shown in **Table. 1**. Subjects were consists of 97 (63%) males and 57 (37%) females. The majority of subjects were aged 5 to 14 years (81 subjects, 52.6%).

Table 1. Subject's characteristics (N=154)

Characteristics		
Gender, n(%)		
Male	97 (63)	
Female	57 (37)	
Age, n(%)		
<1	6 (3.9)	
1- <5	63 (40.9)	
5- <14	81 (52.6)	
>14	4 (2.6)	
Allergens	Positive skin prick test	Parents reported allergy symptoms
Fruit	22	59
Seafood	20	35
Saltwater fish	3	3
Cow's milk	39	66
Chocolate	31	85
Chicken	39	48
Egg	33	53

*one child had more than 1 allergens

From 154 subjects, 59 (38.3%) subjects' parents reported allergy symptoms to fruit. In this study, fruit was represented by orange and tomato extract. Of these 59 subjects, 22 (37.3%) had positive skin prick tests. Of the 95 subjects reported not allergic to fruit, 27 (28.4%) tested positive. In total, there were 49 (31.8%) subjects who tested positive and 105 (68.2%) subjects who tested negative (**Table 2**). Kappa coefficient revealed a very low degree of agreement (0.092) and no statistical significance (P=0.251).

Table 2. Conformity between parental reports and skin prick test results for fruit allergen

Parental report (N=154)	Skin prick test result (N=154)		Total, n (%)	P value
	Positive	Negative		
Positive	22	37	59 (38.3)	0.251
Negative	27	68	95 (61.7)	
Total, n(%)	49 (31.8)	105 (68.2)	154 (100)	

Kappa coefficient=0.092; sensitivity=44.9%; specificity=64.8%; positive predictive value (PPV)=37.2%; negative predictive value (NPV)=71.6%; accuracy=58.4%

Thirty-five (22.7%) subjects had seafood allergies, according to parental reports. Seafood in this study excludes saltwater fish which was tested

separately. Of these 35 children, 20 (57.1%) tested positive. In addition, of the 119 subjects reported to not manifested allergy symptoms, 64 (53.8%) also tested positive. In total, 84 (54.5%) subjects tested positive, while 70 (45.5%) tested negative (**Table 3**). Kappa coefficient revealed a very low degree of agreement (0.022) and no statistical significance (P=0.726).

Table 3. Conformity between parental reports and skin prick test results for seafood allergen

Parental report (N=154)	Skin prick test result (N=154)		Total, n (%)	P value
	Positive	Negative		
Positive	20	15	35 (22.7)	0.726
Negative	64	55	119 (77.3)	
Total, n(%)	84 (54.5)	70 (45.5)	154 (100)	

Kappa coefficient=0.022; sensitivity=23.8%; specificity=78.5%; positive predictive value (PPV)=57.1%; negative predictive value (NPV)=46.2%; accuracy=48.7%

Only 3 (1.9%) subjects were reported allergic to saltwater fish by their parents, and all of them had positive skin prick test. Of the 151 subjects who were reported not allergic to saltwater fish, 76 (50.3%) also tested positive. In total, 79 (51.3%) subjects tested positive and 75 (48.7%) tested negative (**Table 4**). Kappa coefficient revealed a very low degree of agreement (0.037) and not statistical significance (P=0.088).

Table 4. Conformity between parental reports and skin prick test results for saltwater fish allergen

Parental report (N=154)	Skin prick test result (N=154)		Total, n (%)	P value
	Positive	Negative		
Positive	3	0	3 (1.9)	0.088
Negative	76	75	151 (98.1)	
Total, n(%)	79 (51.3)	75 (48.7)	154 (100)	

Kappa coefficient=0.037; sensitivity=3.8%; specificity=100%; positive predictive value (PPV)=100%; negative predictive value (NPV)=49.7%; accuracy=50.6%

Sixty-six (42.9%) subjects were reported allergic to cow's milk by their parents. Of these 66 children, 39 (59.1%) had positive skin prick test. Of the 88 subjects who were reported not allergic, 46 (52.3%) tested positive. In total, 85 (55.2%) subjects tested positive and 69 (44.8%) tested negative (**Table 5**). Kappa coefficient revealed a very low degree of agreement (0.066) and no statistical significance (P=0.400).

Table 5. Conformity between parental reports and skin prick test results for cow's milk allergen

Parental report (N=154)	Skin prick test result (N=154)		Total, n (%)	P value
	Positive	Negative		
Positive	39	27	66 (42.9)	0.4
Negative	46	42	88 (57.1)	
Total, n(%)	85 (55.2)	69 (44.8)	154 (100)	

Kappa coefficient=0.066; sensitivity=45.9%; specificity=60.9%; positive predictive value (PPV)=59.1%; negative predictive value (NPV)=47.7%; accuracy= 52.6%

Eighty-five (55.2%) subjects were reported by their parents to manifest allergic reactions to chocolate. Of these 85 children, 31 (36.5%) tested positive. Of 69 subjects who were reported to not have allergies to chocolate, 22 (31.9%) also tested positive. In total, 53 (34.4%) subjects tested positive to chocolate allergens, while 101 (65.6%) were negative (**Table 6**). Kappa coefficient revealed a very low degree of agreement (0.044) and no statistical significance (P=0.551).

Table 6. Conformity between parental reports and skin prick test results for chocolate allergen

Parental report (N=154)	Skin prick test result (N=154)		Total, n (%)	P value
	Positive	Negative		
Positive	31	54	85 (55.2)	0.551
Negative	22	47	69 (44.8)	
Total, n(%)	53 (34.4)	101 (65.6)	154 (100)	

Kappa coefficient=0.044; sensitivity=58.5%; specificity=46.5%; positive predictive value (PPV)=36.5%; negative predictive value (NPV)=68.1%; accuracy=50.6%

Forty-eight (31.2%) subjects were reported allergic to chicken meat. Of these, 39 (81.3%) had positive skin prick test results. Of the 106 subjects who were reported not allergic, 59 (55.7%) also tested positive. In total, 98 (63.6%) subjects tested positive and 56 (36.4%) tested negative (**Table 7**). Kappa coefficient revealed a low degree of agreement (0.199) but was statistically significant (P=0.02).

For egg allergens, data were collected from 149 subjects. Fifty-three (35.6%) subjects were reported allergic to eggs. Of these, 33 (62.2%) subjects had positive test results. From 96 subjects who were

Table 7. Conformity between parental reports and skin prick test results for chicken allergen

Parental report (N=154)	Skin prick test result (N=154)		Total, n (%)	P value
	Positive	Negative		
Positive	39	9	48 (31.2)	0.02
Negative	59	47	106 (68.8)	
Total, n(%)	98 (63.6)	56 (36.4)	154 (100)	

Kappa coefficient=0.199; sensitivity=39.8%; specificity=83.9%; positive predictive value (PPV)=81.3%; negative predictive value (NPV)=44.3%; accuracy=55.8%

reported to not have allergies, 53 (55.2%) had positive test results. In total, 86 (57.7%) subjects had positive test results and 63 (42.3%) had negative results (**Table 8**). Kappa coefficient revealed a very low degree of agreement (0.062) and no statistical significance (P=0.404).

Table 8. Conformity between parental reports and skin prick test results for egg allergen

Parental report (N=149)	Skin prick test result (N=149)		Total, n (%)	P value
	Positive	Negative		
Positive	33	20	53 (35.6)	0.04
Negative	53	43	96 (64.4)	
Total, n(%)	86 (57.7)	63 (42.3)	149 (100)	

Kappa coefficient=0.062; sensitivity=38.4%; specificity=68.3%; positive predictive value (PPV)=62.3%; negative predictive value (NPV)=44.8%; accuracy=49.4%

Discussion

Food allergy is defined by an abnormal reaction of the immune systems toward certain foods.[5] The reason why some individual show this kind of reaction are not fully understood. Multiple factors may contribute to the condition.[6] In our study, 97 (63%) of 154 subjects were male, similar to previous report recorded by Ebert *et al.*, in 2011. Food allergies, especially to cow's milk are more common in male than female.[7] Boys also tend to have asthma or another atopic diseases more than girls, with ratio 1.8:1.[8]

Allergy prevalence varies worldwide. Discrepant results may be due to different methods, population,[9] geography, and possibly race.[10] An epidemiologic study by *European Academy of Allergy and Clinical Immunology* (EAACI) from 2000 to 2012 recorded a 6.9% prevalence for children aged 0 to 17 years. Allergy prevalence is also tend to be 6 times higher when based on parental reports compared to the real prevalence.[9]

The largest age group of subjects was between 5 to 14 (81 children) year-old. Food sensitization is more common in children because their immature bowel lack protection.[6] Moreover, young age (1-19 years) has been defined as a significant risk factor for food allergies.[8] Allergy incidence was also reportedly decreased with increased age, possibly due to the resolution of several allergies.[11] Children less than 3 years old have the highest risk of disturbed growth and development due to inadequate nutritional intake. Emilination diets should be taken carefully to ensure sufficient nutritional intake.[9]

Positive skin prick test does not always indicate food allergy. It is necessary to connect results to clinical manifestations. However, parents tend to incorrectly deduce the probable food causing the allergy manifestation.[12]

According to Alergologica in 2015, fruits and nuts are the most common food causing allergies in patients aged >5 years.[6] This allergy is common in children and adults.[13] In our study, we found that 22 subjects were diagnosed allergic to fruits, by both parents and skin prick test results. In a Hong Kong study of 352 allergy patients, fruit was on the 4th highest position with 30 (8.5%) reports.[14] Higher results were recorded in a study of 461 people with self-reporting allergy compared to diagnostic test, with fruit in the 1st position with 41.86%.[15]

There were 37 subjects with non-IgE mediated/ food intolerance that clinical manifestations appear similar but negative skin prick test to fruit. These subjects should continue elimination and provocation procedure.[16] Certain fruits such as strawberries, oranges, and tomatoes, are thought to directly stimulate mast cells to release histamine.[17] Subjects with no clinical manifestations but positive skin prick test were sensitized to fruits. A few fruit allergens have similarities to pollens and grass.[12] Fruit allergens have thermolability which can also result in absent clinical manifestation.[18] We found a very low degree of agreement and lack of statistical significance between parental reports and skin prick test results for fruit. Generally, parental reports tend to incorrectly deduce the cause of allergies. Similarly, a study of 78 reports of allergies, only 28 showed positive results by skin

prick test.[12] It is difficult to identify fruit allergies as fruit is often mixed in consumption with other substances.[17]

Seafood is one of eight most common allergens due to IgE-mediated allergy and is a common cause of food allergy anaphylaxis. The allergenicity of seafood is highly affected by processing method.[19] The high incidence of seafood allergy is in line with its high consumption worldwide. This allergy is also a common comorbid for cow's milk allergy.[20] Most seafood allergy manifests in adults.[21] In our study, there was a very low degree of agreement and lack of statistical significance between parental reports and skin prick test results for seafood. Only 20 subjects (57.1%) tested positive out of 35 reported to have seafood allergies by parents. A study of 37 food-allergic children reported that 43.2% were allergic to seafood.[22] Clinical manifestations may also result from hidden ingredients such as food proteins, additives, or parasites.[19] We also noted that 64 subjects (76.2%) showed sensitization without symptoms, which may have resulted from cross-sensitization with house dust mites. House dust mites and seafood share one similar allergen, tropomyosin.[23]

Saltwater fish is a common diet in various communities. Allergies may occur through inhalation, contact, and ingestion.[21] In contrast to cow's milk and egg allergies, fish allergies tend to last for a lifetime and careful diet restriction is needed.[24] The prevalence of fish allergy varies worldwide. In Scandinavian countries, it is ranked 3rd, after egg and milk allergy in infants. In Spain, approximately 30% of 355 children were allergic to fish.[21] However, there were little data on the South East Asian region. In Hong Kong, 0.32% of children aged 2 to 7 years were reported to have fish allergies.[25] In our study, there was a very low degree of agreement and lack of statistical significance between parental reports and skin prick test results for saltwater fish. Only 3 out of 154 subjects' parents reported fish allergies, and all three subjects tested positive. The sensitization rate (positive skin prick test) was also high (79 children, 51.3%), in line with the high consumption of fish in this population. Cross-sensitization between certain fish species, also put individuals at risk for multiple fish allergies.[26]

Cow's milk allergy is most common in children, with a prevalence reaching 2.5%.[27] A study by *Food and Agriculture Organization* (FAO) stated that approximately 90% of children were sensitized and/or allergic to cow's milk and egg.[9] Cow's milk allergy was reported to have the highest food allergy incidence in children aged less than 2 years.[20] Clinically, this allergy commonly appears at age 6 to 12 months, a period in which animal products are introduced.[6] Its prevalence rises every year in parallel with decreased breastfeeding and increased formula feeding.[28] Also, cow's milk allergy has tended to increase in developing countries.[6] We found 39 out of 154 subjects to be positive for allergy to cow's milk, based on both parental reports and skin prick test. Parental reports was used for comparison to skin prick test to determine whether it is Ig-E-mediated allergy. Confirmed diagnosis for cow's milk allergy is necessary because it affects one's quality of life and social participation more than another allergies.[20] Some patients experienced resolution from cow's milk allergy at about one or two years of age,[28] but other children take longer, up to 12 years of age.[7] The mechanism of tolerance is not fully understood until now. In fact, the IgE responses to protein in cow's milk are diverse and no particular structure in cow's milk protein has yet identified as causing allergenicity. Few of recognized allergens such as caseins, ß-lactoglobulin, and a-lactalbumin which is abundant in cow's milk.[28] A high sensitization rate was recorded in our study, as 85 (55.2%) were tested positive out of 154 subjects. Milk from another mammals has similar protein structure and biological properties to cow's milk, resulting in cross-sensitization. Moreover, protein homology between milk from cow, lamb, and goat reaches 80-90%.6 In our study there was a very low degree of agreement and lack of statistical significance between parental reports and skin prick test results for cow's milk.

The consumption of chocolate and its product has risen due to its perceived health advantages, putting people with chocolate allergen sensitivity at risk of allergy. Other ingredients are added to chocolate products, such as milk, fruit, nuts, and sugar,[29] for the purpose of texture, flavor, or nutritional value.[30] Allergy is more common after consumptions of chocolate mixed with other products, than chocolate alone. Hence, food-labelling is crucial.[30] In fact, cocoa seeds were found to be less allergenic than tree nuts, but cross-sensitivity is common. Despite the possibility of cross-sensitivity by other nuts, varied cocoa seed processing methods often denaturized allergenic

proteins, resulting in a low incidence of chocolate allergy.[13] In our study, there was a very low degree of agreement and lack of significance between parental reports and skin prick test results for chocolate.

The prevalence of chicken allergy is uncommon compared to other food such as cow's milk, egg, and fish.3[1] Chicken allergy affects both children and adults, and can appear as primary or secondary allergy. Primarily, sensitization may result from inhalation of bird allergens (in adults) or egg allergies (in children). Secondarily, it might results from cross reaction such as in bird-egg syndrome. Published data on chicken allergy are mostly case reports.[32] In our study, the degree of agreement was low, but statistically significant for chicken. Even though it is a rare allergy, we noted that 48 (31.2%) of 154 subjects were reported to have clinical manifestations, and 39/48 (81.3%) of the reported subjects tested positive. In the case of chicken, careful observation by parents was good. High incidence in this study may also affected by high consumption of chicken, processing methods, and probably through bird exposures.[32]

Egg is also a common cause of food allergies, after milk, in infants and children. Allergy symptoms usually appears after 6 months of age. The initial sensitization could result from exposure to egg in utero or egg protein exposure through breastfeeding.[33] Egg allergies can be caused by consumption of either raw or cooked egg, and egg white or eggyolk. A study indicated that the majority of patients are tolerant with cooked eggs, even though its allergenicity is not only dependent on enzymes and heating. This tolerance can results in small size of wheal in the skin prick test.[34] In our study, egg was ranked 2nd after both chicken meat allergy and cow's milk allergy in first position, with 22.1% out of 154 subject are diagnosed with egg allergy by both parental reports and positive skin prick tests. There was a very low degree of agreement and lack of statistical significance between parental reports and skin prick test results for egg. Egg allergy can also result from inhalaning bird allergens, and known as bird-egg syndrome. These patients are mainly allergic to serum albumin in egg yolk.[34] Prognosis is good, with 80% of children becoming tolerant.[35] However, resolution is slow. A previous study found that half of children with egg allergies developed tolerance by 12 years of age.[36]

Our study had several limitations, as it was retrospective in design and a non-standardized skin prick test procedure may have biased the results. Further studies with prospective methods and a larger number of subjects should be conducted so as to limit unnecessary diet restriction in children.

In conclusion, parental reports of food allergies in children have a low conformity to skin prick test results,. Therefore, we recommend performing skin prick test in every individuals with allergy symptoms.

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Conflict of interest

None declared

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Original Article

Tuberculosis risk factors in children with smear-positive adults in the household

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Abstract

Background Children in household contact of adults with smear-positive tuberculosis (TB) are at higher risk of TB infection. Screening of these children is a main strategy for eliminating childhood TB.

Objective To determine risk factors of TB among children in household contact with smear-positive adult TB patients.

Methods This case-control study was conducted in 5 public health centers at Batu Bara District, North Sumatera. We studied children from birth to 18 year-old living in the same house as adults with smear-positive TB. A tuberculosis scoring system was used to diagnosis TB in the children. Associations between risk factors and the incidence of TB were analyzed using Chi-square, Mann-Whitney U, and logistic regression tests.

Results We enrolled 145 children who had household contact with smear-positive adult TB patients. Subjects were allocated to either the case group [TB score >6; 61 subjects (42.0%)] or the control group [TB score <6; 84 subjects (58.0%)]. Bivariate analysis revealed that nutritional status, immunization status, number of people in the house, sleeping in the same bed, and duration of household contact had significant associations with the incidence of TB. By multivariate logistic regression analysis, nutritional status and duration of household contact were significant risk factors for TB, with OR 5.89 and 8.91, respectively.

Conclusion Malnutrition and duration of household contact with smear-positive adult TB patients of more than 6 hours per day are risk factors for TB among children. **[Paediatr Indones. 2018;58:66-70; doi: http://dx.doi.org/10.14238/pi58.1.2018.66-70]**.

Keywords: *risk factors; tuberculosis; household contact; smear-positive*

Mycobacterium tuberculosis infection in childhood continues to be a worldwide problem because of its high prevalence.[1] Childhood TB infection has a limited influence on TB epidemiology. However, children with TB infection could contribute to future adult TB cases.[2] Tuberculosis is a major cause of morbidity and mortality in all age groups worldwide.[3]

Childhood TB carries a large proportion of the overall TB burden, representing 15 to 40% of all cases and causing more than 10% of pediatric hospital admissions and deaths. To limit TB incidence, the US and European countries provide preventive treatment for people with TB infection.[2] The aim of this study was to determine risk factors of TB among children with smear-positive TB adult patients in the household.

This study was presented at the *Pertemuan Ilmiah Tahunan Ilmu Kesehatan Anak VII/PIT IKA VII* (The 7th Annual Scientific Meeting for Child Health), Surabaya, November 2-4, 2015.

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Methods

This case-control study was conducted from January to March 2015 in 5 public health centers at Batu Bara District, North Sumatera. We studied children from birth to 18 years, who lived in the same house as smear-positive TB adults. A tuberculosis scoring system was performed to diagnosis TB in this study (**Table 1**).4 Children with TB scores below 6 were classified into the control group, while children with TB scores of 6 or above were classified into the case group. Associations between risk factors and the incidence of TB were analyzed using Chi-square, Mann-Whitney U, and logistic regression tests.

Table 1. Tuberculosis scoring system[4]

Features \ Scoring	0	1	2	3	Score
Contact	Not clear	-	Reported, AFB (-)	AFB (+)	
Tuberculin skin test	-	-	-	Positive	
Body weight	-	< red line, decreased BW	Severe malnutrition	-	
Fever	-	Unexplained	-	-	
Cough	< 3 weeks	≥ 3 weeks	-	-	
Node enlargement	-	≥ 1 node, ≥ 1 cm, painless	-	-	
Bone joint	-	Swelling	-	-	
Chest x-ray	Normal	Sugestive	-	-	
				Total score	

AFB=acid fast bacilli; body weight on *Kartu Menuju Sehat*/KMS (local growth chart)

Data was processed using *SPSS* software. Chi-square and Mann-Whitney U tests were used to determine risk factors of TB among children with smear-positive TB adults in the household. Bivariate analysis was performed first. Variables with P values < 0.25 were further subjected to multivariate logistic regression analysis. Results were considered to be statistically significant for P values > 0.05, with 95% confidence intervals. The study was approved by the Ethics Review Committee of University of Sumatera Utara Medical School.

Results

We enrolled 145 children who had household contact with smear-positive adult TB patients, comprising 61 (42.0%) cases and 84 (58.0%) controls.

The mean age of subjects was 5.7 (SD 3.21) years in the case group and 5.5 (SD 2.66) years in the control group. Distribution of males and females was similar between groups. The majority of subjects in both group had BCG immunization. The majority of subjects in the case group had malnutrition, but that of the control group had normal nutritional status (**Table 2**).

Table 3 shows that nutritional status was associated with the incidence of TB. A significantly greater percentage of children in the case group had malnutrition than in the control group [51 (83.6%) vs. 17 (20.2%), respectively, (P=0.0001)]. Number of

Table 2. Characteristics of subjects

Characteristics	Case (n=61)	Control (n=84)
Sex, n (%)		
Male	33 (54.0)	41 (48.8)
Female	28 (45.9)	43 (51.8)
Mean age (SD), years	5.78 (3.21)	5.5 (2.66)
Nutritional status, n (%)		
Malnutrition	51 (83.6)	17 (20.2)
Normal	10 (16.3)	67 (79.7)
BCG scar, n (%)		
No	24 (39.3)	11 (13.0)
Yes	37 (60.6)	73 (86.9)
Index cases, n (%)		
Father	26 (42.6)	30 (49.1)
Mother	50 (59.5)	21 (25)
Other	5 (8.1)	13 (15.4)

Table 3. Bivariate analysis of characteristics of subjects and tuberculosis

Characteristics	Case (n=61)	Control (n=84)	OR	95% CI	P value
Nutritional status, n (%)					
Malnutrition	51 (83.4)	17 (20.2)	3.81	1.88 to 7.74	0.0001
Normal	10 (16.3)	67 (79.7)			
BCG scar, n (%)					
No	24 (39.3)	11 (13.0)	2.03	1.44 to 2.87	0.01
Yes	37 (60.6)	73 (86.9)			
Number of people in the house, n (%)					
> 6	44 (72.1)	26 (30.9)	2.59	1.89 to 3.55	0.01
≤ 6	17 (27.9)	58 (69.1)			
Sleeping in the same bed , n (%)					
Yes	31 (50.8)	9 (10.7)	2.71	1.92 to 3.83	0.001
No	30 (49.1)	75 (89.2)			
Duration of contact, n (%)					
> 6 hours/day	45 (73.7.9)	8 (9.5)	4.88	3.08 to 7.70	0.0001
≤ 6 hours/day	16 (26.2)	76 (90.4)			

people in the house was significantly associated with TB incidence (P=0.0001), as 72.1% of the case group lived with more than 6 people in the house. Sleeping in the same bed was also significantly associated with TB incidence (P=0.001), and raised the risk of TB infection by 2.7 times (OR 2.713; 95%CI 1.92 to 3.83). The immunization BCG status was associated with incidence of TB. This study showed that 73 children (86.9%) in the control group had received BCG immunization. Duration of contact with adult TB patients was also significantly associated with incidence TB (P=0.0001). There were 45 children (73.8%) in the case group who had >6 hours/day of close contact with adult TB patients.

Table 4 shows that the variables significantly associated with incidence of TB were nutritional status and duration of contact, as revealed by multivariate analysis. Longer duration of contact per day was the highest risk factor associated with TB cases (OR 8.91).

Table 4. Multivariate analysis of risk factors for TB

Risk factors	OR (95% CI)	P value
Nutritional status	5.88 (1.48 to 23.40)	0.012
BCG status	2.22 (0.79 to 6.21)	0.118
Number of people in the house	1.98 (0.82 to 6.01)	0.12
Sleeping in the same bed	2.72 (0.76 to 9.78	0.110
Duration of contact	8.91 (2.58 to 30.75)	0.001

Discussion

In this study, we examined environmental characteristics associated with the risk of tuberculosis in children who lived with smear-positive adult TB patients. A previous study showed that environmental characteristics pose a risk of tuberculosis in children who live with adult TB patients.[5] A Surabaya study showed that the number of family members in the house and houses with poor sanitation were risk factors of tuberculosis incidence.[5] In our study, a history of contact with the index case of more than 6 hours per day increased the risk of tuberculosis by 8 times.

Another study assessed the risk factors of TB infection in children with adult TB patients in the household and found a TB infection incidence of 24-69% and a TB disease case incidence of 3.3-5.5%.[6] In addition, Loredo *et al.* showed that the prevalence of TB in children with smear-positive adults in the household was 2-2.7%.[7] Several studies have indicated that close contact with adult TB patients raise the risk of becoming infected.[8,9] *The World Health Organization* (WHO) recommends that all children aged ≤ 5 years living with a smear-positive adult TB patient be screened.[10] Screening this population of children is one strategy to eliminate TB.[11] About 10% of children with latent TB infection develop signs and symptoms of TB disease.[10] The TB assessment of children who have contact with adult TB patients was done by tuberculin skin test and chest radiography.[12]

Children under 5 years of age have a greater

risk to develop from infected TB to TB disease because their immune system has not fully developed. T lymphocyte-mediated immunity is cellular immunity. T lymphocytes consist of T lymphocyte memory cells and effector T lymphocytes. Memory T lymphocytes consist of CD4 and CD8 memory lymphocytes. Effector T lymphocytes consist of cytotoxic T lymphocytes which lysis target cells and effector T CD4 which activate macrophage, B cells and other cells.[11] If the cellular immunity has been formed, newly TB organism will be demolished by cellular immunity.[12]

Nutritional status had a significant association with TB incidence. Evidence has suggested that malnutrition affects genetic expression and immune function, which are predisposing factors for tuberculosis progression.[9] We found a significant association between malnutrition and TB.

The thymus has an important role in the maturation of T lymphocytes and requires proper nutrition in the perinatal period and early childhood. Thymus atrophy in children with malnutrition was associated with increased of infant mortality due to infection. Protein energy malnutrition decreased the size of the thymus and cortical thymocyte apoptosis, changed the microenvironment surrounding lymphoid tissue and epithelial cells, as well as decreased hormone production and proliferation of thymocyte thymulin. Zinc deficiency also contributed to dysfunction of the thymus.[13]

High humidity and poor ventilation can increase the risk of TB. Moreover, a high population density in a housse and a large number of household members increase the infection rate spread from active adult TB to children. Children living with tuberculous adults may develop TB disease depending both on the closeness of the contact and the concentration of the Mycobacterium tuberculosis agent in the sputum index.[14]

This study had strengths and limitations. The limitation of this study was that it was the first study to track children with a history of contacts with adult patients with smear-positive sputum in Batubara, that's why make us difficult to investigaste children, especially for sputum examination in diagnosing tuberculosis in children. Sputum examination is a tool for diagnosing tuberculosis in Batubara, but obtaining sputum from children remains a challenge. Hence, we used the less precise TB scoring system to define active TB disease in our subjects. Other limitations were not assessing the index case, such as by chest X-ray examination, the state of the home environment, such as sanitation and house size, or the socioeconomic status of the family. Chest X-rays of the index cases may be used to assess the severity and location of abnormalities in the lungs.[15] Extensive infiltrates and cavities in the upper lobe were risk factors in the transmission of tuberculosis from adults to children.[12] Adverse environmental conditions such as poor ventilation, humid housing conditions, and the number of occupants in the home may also increase tuberculosis incidence.[14]

In conclusion, malnutrition and duration of household contact with adults having smear-positive TB for more than 6 hours per day are risk factors for TB among children who reside with an adult TB patient.

Conflict of interest

None declared.

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Original Article

Hematological parameters and remission induction of childhood acute lymphoblastic leukemia

Roro R. W. Perdani, Bambang Sudarmanto

Abstract

Background High-risk acute lymphoblastic leukemia (ALL) is one of the most common childhood malignancies in Indonesia. Many factors can inhibit the induction of remission. Hematological parameters are usually not normal. Identification of corresponding factors is important to increase the likelihood of successful inductions.

Objective To assess for associations between hematological parameters and induction of remission in children with acute lymphoblastic leukemia.

Methods Data were collected from medical records of ALL patients hospitalized in the Pediatric Ward at Dr. Kariadi Hospital from May 2014 – May 2016. Dependent variables were hemoglobin, leukocytes, platelets, and absolute neutrophil count (ANC) levels; the independent variable was induction of remission.

Results Out of 55 patients, 33 (60%) had anemia, 6 (10.9%) had leukocytosis, and 1 (1.8%) had hyperleukocytosis, whereas 9 (34.5%) had leukopenia and 29 (52,7%) had normal leukocyte levels. Thirty-one subjects (56.4%) had thrombocytopenia, 15 (27.3%) had thrombocytosis, and only 9 (16.4%) patients had normal platelet counts. There were 29 (52.7%) with ANC > 500, whereas 26 (47.3%) had ANC level ≤ 500. Most patients (80%) experienced remission induction, while 20% did not. There were significant associations between ANC level and induction of remission (P=0.010) as well as between platelet level and induction of remission (P= 0.033). Regression logistic test revealed that ANC level ≤ 500 was associated with a 7-fold lower remission event compared to ANC level > 500 (RR 7.147; 95%CI 1.38 to 37.14).

Conclusion Lower ANC level (≤ 500) is significantly associated with lower remission compared to higher ANC level (> 500). **[Paediatr Indones. 2018;58:71-4; doi: http://dx.doi.org/10.14238/pi58.1.2018.71-4]**.

Keywords: *high-risk ALL; remission; induction; hematological parameters*

Leukemia is the most common malignancy in childhood.[1] From an epidemiological standpoint, acute leukemia occurs in 30%-40% of all childhood malignancies.[2-4] Standard and high-risk groups are widely known to have different outcomes, therapy, and even prognoses. The high-risk group has been linked to poor prognosis. High-risk ALL chemotherapy consists of the induction phase, consolidation, re-induction, and maintenance therapy, with each phase having its own goals. The main induction phase achievement in ALL chemotherapy is remission.[5-7] A remission failure could be caused by abnormal hematologic parameters, such as initial hemoglobin level, initial leukocytes and platelets, as well as low ANC level.[8] Identifying the corresponding factors is very important in order to increase the likelihood of remission.

Methods

This retrospective cohort study was conducted from May 2014 through May 2016. The inclusion criteria

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were children between 0-18 years of age who were diagnosed with ALL at Dr. Kariadi Hospital, Semarang, had never undergone any Indonesian 2006 ALL chemotherapy protocols, and agreed to participate in the study. Hematologic parameters consisted of initial of hemoglobin, leucocyte, and platelet levels, and ANC level. Initial hemoglobin level was categorized anemic and non-anemic according to WHO criteria on age. Whereas initial leucocyte level was divided into normal (10,000 - < 50,000/mm^3), leukopenia (< 10,000/mm^3), leukocytosis (50,000-100,000/mm^3) and hyperleucocytosis (> 100,000/mm^3). The other parameter was initial platelet count which categorized into normal (150,000-400,000/mm^3), thrombocytopenia (< 150,000/mm^3) and thrombocytosis (> 400,000/mm^3). The outcomes of induction was remission and no remission. Remission was defined with the blast cell in bone marrow puncture 20%, while no remission was if the blast cell ≤ 20%. The diagnosis of high risk ALL was made using the *National Cancer Institute* (NCI) criteria: peripheral leukocyte count > 50,000/mm^3, children aged <1-year-old or ≥ 10-year-old, evidence of mediastinal mass, or blast cell absolute count in peripheral blood ≥ 1,000/mm^3. Patients lost to follow up or who died before undergoing induction therapy were excluded from this study.

The standard risk group was aged between 1 and 10 years and the high risk group was aged younger than 1 year or older than 10 years.

Data were collected from subjects' medical records. The dependent variables were hematologic parameters, consisting of initial hemoglobin level, initial leukocyte and platelet levels, and ANC; the independent variable was remission induction. Chi-square test or Fisher's exact test were used for bivariate analysis and relative risk; logistic regression test was used for multivariate analysis. A P value of < 0.05 is considered significant. This study was approved by the Research Ethics Committee of the Diponegoro University Medical School.

Results

During the 2 year study period, 55 children met the inclusion criteria. Baseline characteristics are shown in **Table 1**. Most subjects were in the standard risk group

Table 1. Baseline characteristics of subjects

Characteristics	n (%)	Mean (SD)	Median (range)
Age		6.96 (3.62)	6.58 (0-17.75)
Standard risk (1 to <10 years)	45 (81.8)		
High risk (<1 year or ≥10 years)	10 (18.2)		
Sex		8.91 (2.51)	9.4 (2.2-13.3)
Male	34 (61.8)		
Female	21 (38.2)		
Initial haemoglobin levels			
Anemic	33 (60)		
Non-anemic	22 (40)		
Initial leukocyte levels		39,944.24 (60,876.69)	9,000 (900-312,700)
Normal	29 (52.7)		
Leukopenia	19 (34.5)		
Leukocytosis	6 (10.9)		
Hyperleukocytosis	1 (1.8)		
Initial platelet counts		84,594.36 (101,068.48)	39,000 (190 – 352,000)
Normal	9 (16.4)		
Thrombocytopenia	31 (56.4)		
Thrombocytosis	15 (27.3)		
ANC count			
≤ 500	26 (47.3)		
> 500	29 (52.7)		
Induction outcomes			
Remission	44 (80)		
No remission	11 (20)		

(81.8%) and most were male (61.8%). Our subjects' median age was 6.58 years when diagnosed.

In the bivariate analysis, two factors were significantly associated with worse remission rates in the induction phase: platelet levels (thrombocytopenia) and ANC level <500 (**Table 2**). Further analysis by logistic regression test revealed that ANC level ≤500 was associated with a 7-fold lower remission event compared to ANC level >500 (RR 7.147; 95%CI 1.38 to 37.14) (**Table 3**).

Table 2. Bivariate analysis factors associated with induction of remission

Variables	Outcomes, n(%)		P value
	Remission (n=44)	No remission (n=11)	
Anemia			1.000
Yes	26 (59.1)	7	
No	18 (40.9)	4	
Leukocyte levels			0.122
Normal	26 (59.1)	3	
Leukopenia	14 (31.8)	5	
Leukocytosis	3 (6.8)	3	
Hyperleukocytosis	1 (2.3)	0	
Platelet levels			0.033
Normal	9 (20.5)	0	
Thrombocytopenia	21 (47.7)	10	
Thrombocytosis	14 (31.8)	1	
ANC count			0.010
> 500	27 (61.4)	2	
≤ 500	17 (38.6)	9	

Table 3. Multivariate analysis of factors associated with induction of remission

Variable	RR	95%CI	P value
ANC level	7.147	1.38 to 37.14	0.019

Discussion

In our study of 55 subjects, remission in the induction phase was achieved in 44 subjects (80%). Reports from developed countries also show remission rates in the induction phase to be approximately 80%.[6,7] A Busan, Korea study found that 43 (95.6%) patients achieved complete remission.[9] Our subjects' median age was 6.58 years when diagnosed, with an age range of 0-17.8 years. A previous study noted the mean age of subjects was 5.4 years at the time of diagnosis, with a range of 2 – 12 years.[10] The standard risk group comprised of 45 (81.8%) subjects, whereas the high risk group had 10 (18.2%) subjects, which consisted of 1 subject younger than 1 year and 9 subjects older than 10 years. Another study in Cipto Mangunkusumo Hospital, Jakarta, demonstrated different result of age groupings to those of our study, where most patients were between 2 and 10 years of age (59%).[11] In our study, 61.8% of subjects were male and 38.2% were female. Similarly, the distribution of male and female subjects in a Makassar study was 60% and 40%, respectively.[12]

Subjects' mean hemoglobin level was 8.91 g/dL (SD 2.51), and the median was 9.4 (range 2.2-13.3) g/dL. In a Korean study, the the median hemoglobin level was a similar 8.5 (range 3.2-14.7) g/dL.[9]

In our study, the majority of subjects (52.7%) had normal leukocyte levels. The remaining subjects had 34.5% leukopenia, 10.9% leukocytosis and 1.8% hyperleukocytosis. In contrast, a Surabaya study reported that 23.2% of subjects had leukocytosis, with leukocyte level of > 50,000/mm^3. Leukopenia was observed in 30% of their subjects.[5]

The majority of our subjects (56.4%) had thrombocytopenia, with mean platelet count of 84,594.36/mm^3. Again, our results differed from the Surabaya study. In their high risk ALL patients, the highest platelet levels were 50,000-100,000/mm^3, comprising 42.8% of the total subjects.[5]

Bivariate analysis showed that the ANC level ≤ 500 (P=0.010) and thrombocytopenia (P=0.033) were significantly associated with remission in the induction phase. In contrast, a study from Korea noted that initial leukocyte level was associated with event free survival, and high levels of leukocytes (10 x 10^9/L) could worsen the event free survival (EFS) (P<0.001).[9]

In conclusion, the study shows that ANC level of ≤ 500 is significantly associated with worse remission rate. The ANC level has been used to diagnose neutropenia. Patients with neutropenia are susceptible to infection and febrile neutropenia may occur.[13,14] In our subjects, it is possible that the association between ANC level ≤ 500 and worse remission rates was caused by severe infection that inhibited the remission by the end of induction phase. However, we did not analyze for an association between febrile neutropenia and remission in the induction phase.

Conflict of Interest

None declared.

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Original Article

Consanguinity and congenital heart disease in offspring

Aris Fazeriandy, Muhammad Ali, Johannes H. Saing, Tina Christina L. Tobing, Rizky Ardiansyah

Abstract

Background High-risk acute lymphoblastic leukemia (ALL) is one Background Congenital heart disease (CHD) is a common congenital abnormality in children. Consanguineous marriage has been identified as a risk factor of CHD. There was an autosomal recessive pattern of inheritance seen in children with some forms of congenital heart disease.

Objective To assess the possible association between consanguineous marriage and congenital heart disease incidence in the offspring.

Methods A case-control study was conducted from March to May 2016 on pediatric patients at H. Adam Malik General Hospital, Medan. Subjects were allocated into two groups, 100 children with CHD in the case group, and the rest in the control group. Data were analyzed using Chi-square and logistic regression tests. In the present study, P value less than 0.05 was considered statistically significant.

Results In the case group, 14 patients (14%) were born of consanguineous marriages. In the control group, only 5 patients (5%) were born of consanguineous marriages. There was a significant association between consanguineous marriage and CHD (OR 1.551; 95%CI 1.138 to 2.113). Based on the result of multivariate analysis, consanguineous marriage was a risk factor for CHD in offspring (Wald=4.525; P=0.033).

Conclusion Consanguineous marriage is a risk factor for CHD in offspring. **[Paediatr Indones. 2018;58:75-9; doi: http://dx.doi.org/10.14238/pi58.1.2018.75-9]**.

Keywords: *consanguineous marriage; congenital heart disease; offspring*

Congenital heart disease (CHD) is the most common congenital malformation found in children.[1,2] The worldwide incidence of CHD has held constant at about 8-10 per 1,000 live births.[1,3,4] However, that number is higher if the parents were blood-related (consanguineous marriage).[5] Worldwide, consanguineous marriage increases the risk of CHD phenotype occurrence by 2 to 3-fold.[6] The role of consanguinity in the etiology of CHD is supported by studies of blood-related marriage, which show an autosomal recessive pattern in some CHDs.[7]

Consanguineous marriage is a marriage between two people descended from the same ancestor, and is still common in some regions of the world.[8,9] It often has genetic implications for the offspring.[8,10-12] From the genetical point of view, marriages between couples who have a biologic relationship as second cousin or closer are considered consanguineous (originated from

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Latin consanguineus, which means having the same blood relation).[13]

A case-control study in Pakistan found that 49% of CHD cases were related to consanguineous marriage.[14] In Lebanon, 17.9% of CHD cases were related to consanguineous marriage, particularly between close cousins.[15] In Indonesia, 12 CHD cases had a family history of CHD, however, the authors did not assess the history of consanguineous marriage in the study.[16] Based on prior studies, we aimed to assess for a relationship between consanguineous marriage and CHD incidence in Indonesian children.

Methods

We did an observational, analytic, case-control study to assess consanguinity as a risk factor for CHD incidence in children. It was conducted in the Cardiology Division of the Department of Child Health, H. Adam Malik Hospital, Medan between March 16 and June 16, 2016. Subjects were allocated into two groups by consecutive sampling, 100 children with CHD in the case group, and 100 children without CHD in the control group. Echocardiography was done on all patients. The inclusion criteria were 0-18 years old children, with or without CHD. Exclusion criteria were children with acquired heart disease, patients with the evaluated risk factors, i.e., family history of CHD, maternal drug use, and maternal comorbidities.

Data were analyzed to assess for the relationship between consanguineous marriage with CHD incidence using Chi-square for normally distributed data and Fischer's exact test for abnormally distributed data. Odds ratio were obtained from 2x2 table (with 95%CI). Univariate logistic regression analysis was done for CHD types, demography characteristics, and risk factor profile, for the case and control group. Multivariate analysis was done to obtain independent predictor. A P value of <0.05 was considered statistically significant. We used *SPSS 15* software for data analysis.

Written informed consent form was obtained from the parents of the study subjects. This study was approved by Ethical Committee of the University of Sumatera Utara Medical School.

Results

The total sample number was 200 children, with a mean age of 4.3 (SD 4.6) years. The mean birth weight and length were 3,143.5 (SD 305.8) grams and 48.1 (SD 1.2) cm, respectively. All children were delivered full term, with mean gestational age of 38.6 (SD 0.9) weeks. Demographic characteristics of children are shown in **Table 1**.

In the case group, 14 patients (14%) were born of consanguineous marriages. In the control group,

Table 1. Subjects' characteristics

Characteristics	With CHD (n=100)	Without CHD (n=100)
Gender, n (%)		
Male	52 (52)	49 (49)
Female	48 (48)	51 (51)
Paternal ethnicity, n (%)		
Acehnese	16 (16)	15 (15)
Bataknese	45 (45)	30 (30)
Javanese	14 (14)	22 (22)
Melayu	16 (16)	27 (27)
Others	9 (9)	6 (6)
Maternal ethnicity, n (%)		
Acehnese	13 (13)	14 (14)
Bataknese	39 (39)	26 (26)
Javanese	29 (29)	41 (41)
Melayu	10 (10)	16 (16)
Others	9 (9)	3 (13)
Religion, n (%)		
Islam	73 (73)	75 (75)
Protestant	18 (18)	17 (17)
Catholic	9 (9)	8 (8)
Consanguineous marriage, n (%)		
Yes	14 (14)	5 (5)
No	86 (86)	95 (95)
Family history of CHD, n (%)		
Yes	3 (3)	1 (1)
No	97 (97)	99 (9)
Maternal drug use, n (%)		
Yes	0 (0)	0 (0)
No	100 (100)	100 (100)
Maternal comorbidities, n (%)		
Yes	5 (5)	6 (6)
No	95 (95)	94 (94)
CHD type, n (%)		
No CHD	0 (0)	100 (100)
Atrial septal defect	25 (25)	0 (0)
Hypoplastic right ventricle	2 (2)	0 (0)
Patent ductus arteriosus	24 (24)	0 (0)
Ventricular septal defect	30 (30)	0 (0)
Transposition of great arteries	6 (6)	0 (0)
Tetralogy of Fallot	12 (12)	0 (0)
Pulmonary stenosis	1 (1)	0 (0)

only 5 patients (5%) were born of consanguineous marriages. Chi-square test showed a statistically significant relationship between consanguineous marriage and CHD incidence in the offspring (OR: 1.551) (**Table 2**).

Logistic regression test was done to assess the effect of the risk factors of CHD, such as consanguineous marriage, family history of CHD, and maternal comorbidities. **Table 3** shows consanguineous marriage as the only risk factor of CHD occurrence (Wald=4.525; P=0.033).

Table 2. Relationship between consanguineous marriages ith CHD incidence

	Congenital heart disease		OR	95%CI
Consanguineous marriage, n(%)				
Yes	14 (14)	5 (5)	1.551	1.138 to 2.113
No	86 (86)	95 (95)		

Table 3. Risk factors of CHD

Risk factors	Constant	Wald	P value*
Consanguineous marriage	1.154	4.525	0.033
Family history of CHD	1.214	1.085	0.298
Comorbidity	-0.169	0.072	0.789

*logistic regression test

Discussion

Out of 100 cases of CHD and 100 controls, 19 children were offsprings of consanguineous marriages, which were 14% of cases and 5% of controls. Similar results were reported from several countries such as Iraq,[4] Saudi Arabia,[8] Pakistan,[14] Iran,[9,18] China,[17] India,[19] Lebanon,[20] and Canada.[21] The proportions varied among countries. We found a significant relationship between consanguinity and the risk of CHD occurrence in the offspring. The relationship of this study with the precedings is that they have similar charateristics such as demography, religion, and culture. However, we did not assess the level of relationship in the consanguineous marriages, nor the genes contributing to the risk.

Consanguinity is a significant risk factor for CHD incidence. The most common types of CHD in all population are ventricular septal defect, atrial septal defect, and tetralogy of Fallot.[22] Our study found that the CHDs found in children from consanguineous marriage were atrial septal defect (25%), persistent ductus arteriosus (24%), ventricular septal defect (30%), hypoplastic right ventricle (2%), and transposition of the great arteries (6%). Those proportions were similar to a study in Iraq which stated that the most common CHD was of the non-cyanotic type, which was atrial septal defect (66.6%). Other cases were ventricular septal defect, persistent ductus arteriosus and transposition of the great arteries.[4] Also, a study in United Arab Emirates found atrial septal defect (49%) as the most common type of CHD.[8] A South Indian study found that the most commonly found CHDs were atrial septal defect and persistent ductus arteriosus.[23] However, our study contrasted from these studies that we did not assess the level of relationship in the consanguineous marriages, e.g., first cousins, while this was assessed in the previous studies in Iraq, Saudi Arabia, and South India.

In contrast, a Pakistani study reported that the CHD cases had the following types: ventricular septal defect (97 cases), persistant ductus arteriosus, atrial septal defect, pulmonary stenosis, tetralogy of Fallot, transposition of great arteries, and hypoplastic right ventricle.14 A similarity in our study was that non-cyanotic CHDs were more commonly found than cyanotic CHDs, with transposition of the great arteries as the most common cyanotic CHD found in both studies. An Egyption study reported CHD cases with ventricular septal defect and atrial septal defect, accompanied by dysmorphic features from chromosome disorders trisomy 21 and trisomy 14.[22] In our study, no dysmorphic features were found in the study subjects. A Lebanese study had CHD cases of tetralogy of Fallot, valvar aortic stenosis, and atrial septal defect.[20]

In this study, there was a slightly higher incidence of CHD in males. This finding is consistent with several studies, such as in Iraq,[4] Saudi Arabia,[8] Pakistan,[14] and Lebanon.[20] In contrast, a study in China reported that CHD was more prevalent in

girls.[17] Overall, there was no significant difference in the occurrence of CHD between the two sexes.

In this study, the majority of CHD cases were found in Muslims. This might be due to the fact that the majority of Indonesia's population is Muslim. Furthermore, the reason for consanguineous marriage is to tighten the relationships and kinships between relatives. As a result, the practice of matchmaking between family relatives persists. Ethnic relations and cultural practices influence the occurrence of consanguineous marriage. Most patients who visited the Cardiology Department of Haji Adam Malik Hospital came from the province of Aceh and North Sumatra, where customs, regulations, and culture strongly influence the society. However, the ethnic group that dominates visits to the Cardiology Department is Bataknese, from both the father and the mother. The occurrence of consanguineous marriage is higher in the Batak population due to cultural influences such as marga (clan), pariban, and impal. However, consanguineous marriage is more likely to occur in rural areas compared to urban areas, where people have more opportunity for education and wider options for finding a spouse. Lack of knowledge and education about the risk factors of CHD are obstacles that increase the risk of CHD occurrence. Thus, genetic counseling in rural areas is needed to decrease the morbidity and mortality of CHD cases. Our study is consistent with studies in Saudi Arabia,[8] Iran,[9] Pakistan,[14] and Lebanon,[20] with majority Muslim populations. However, in India and South India the majority of people are Hindu. A study in Canada showed that CHD was mostly found in migrants from the Middle East.[21]

According to studies in Pakistan[14] and South India,[23] not all consanguineous marriages put offspring at risk for CHD. This finding is consistent with our results, in which there were five (2.5%) children from consanguineous relationships who did not develop CHD. In this study, other risk factors for CHD were evaluated. There were three CHD cases with a family history of CHD, without consanguineous marriages. This finding is consistent with a study in Indonesia which reported persistent ductus arteriosus to be the most prevalent, and without consanguineous marriage.[16] It is also found in other studies, such as in Pakistan (14%),[14] China (4.6%),[17] Canada (19%),[21] and Egypt (13%).[22] In this study, comorbid risk factors of the occurrence of CHD were found in 2.5% of cases, i.e., hypertension during pregnancy, but it was not statistically significant. This finding is consistent with research in Pakistan where 14% of CHD cases were associated with maternal hypertension and diabetes during pregnancy.[14] In our study, we found no significant relationship between maternal use of drugs and the risk of CHD in children. The Pakistani study found maternal use of drugs in 2% of cases.[14]

Genetic mutations associated with CHD can now be detected. It should be noted that a wide variety of genes are involved in the process of cardiogenesis.3 With the rapid development of science, the types of genes in each individual can be detected by single nucleotide polymorphisms (SNPs). An Egyptian study proposed an autosomal recessive role as a cause of CHD in children associated with consanguineous marriage, with the exception of patent ductus arteriosus. We used echocardiography for the diagnosis of CHD and conducted interviews to find a history of consanguineous marriage. However, evaluation of the genetic abnormalities from the SNPs in the 19 cases of CHD and consanguinity was not feasible due to the lack of available equipment. The role of autosomal recessive inheritance on the occurrence of CHD in children in this study cannot be ruled out. Therefore, further research is needed.

Limitations of this study were the small sample size and lack of genetic evaluation in the form of SNPs. In conclusion, consanguineous marriage is a risk factor for congenital heart disease in the offsprings.

Conflict of interest

None declared.

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Original Article

Diabetes duration and thyroid stimulating hormone levels in children with type 1 diabetes mellitus

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Abstract

Background Children with type 1 diabetes mellitus (T1DM) are at risk of thyroid dysfunction. An association between diabetes duration and thyroid stimulating hormone level remains inconclusive.

Objective To assess for a possible association between diabetes duration and thyroid stimulating hormone levels in children with T1DM.

Methods We conducted a cross-sectional study from January to June 2017 in the Pediatric Endocrine Outpatient Clinic at Dr. Soetomo Hospital. Subjects were children with T1DM aged 7 to <18 years. Exclusion criteria were children with diabetic ketoacidosis, previously diagnosed thyroid problems, and hospitalization in the pediatric intensive care unit (PICU).

Results From the 55 regular patients in our outpatient clinic, 34 patients were included in the study. Nineteen (54.3%) subjects were male, and the overall mean age was 11.3 years. Subjects' mean duration of diabetes was 3 years and their mean thyroid stimulating hormone concentration was 3.76 mIU/L. Pearson's correlation test revealed no significant association between duration of diabetes and thyroid stimulating hormone level (rs=-0.068; P=0.703).

Conclusion There is no significant association between duration of diabetes and thyroid stimulating hormone levels in children with T1DM. **[Paediatr Indones. 2018;58:80-3; doi: http://dx.doi.org/10.14238/pi58.1.2018. 80-3]**.

Keywords: *diabetes duration; thyroid stimulating hormone; type 1 diabetes mellitus children*

According to the *World Health Organization* (WHO) and the *International Diabetes Federation* (IDF), the prevalence of diabetes is on the rise.[1,2] Data from the *Pediatric Endocrine Working Group, Indonesian Pediatric Society*, showed that 1,153 patients suffered from T1DM until April 2016.[3] Thyroid dysfunction is reportedly higher among T1DM patients.[4-9] Among diabetic adult populations, 15-30% reported autoimmune thyroiditis compared to 5-22% in children.[8] In the non-diabetic population, 2-10% adults and 1-4% children reportedly have the condition.[10,11] To date, serum thyroid stimulating hormone levels in T1DM patients have rarely been studied in Indonesia. Therefore, the objective of this study was to assess for a possible association between diabetes duration and thyroid stimulating hormone concentration in children with type 1 diabetes mellitus.

Methods

This cross-sectional study was carried out from January to June 2017 in the Pediatric Endocrine Outpatient

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Clinic (OPC) at Dr Soetomo Hospital, Surabaya, East Java. Subjects were children with T1DM aged 7 to <18 years. Exclusion criteria were diabetic ketoacidosis, previously diagnosed thyroid problems, and hospitalization in the PICU. Blood specimens were processed by an ADVIA Centaur immunoassay system, using an electrochemilluminescence immunoassay (ECLIA) method to measure TSH levels. Statistical analysis was done with Pearson's correlation test. Results with P values <0.05 were considered to be statistically significant. FT4 levels were obtained for subjects with abnormal TSH levels.

Table 1. Baseline characteristics of subjects

Characteristics	(N=34)
Male sex, n (%)	19 (55.9)
Mean body weight (SD), kg	33.34 (12.6)
Mean height (SD), cm	136.6 (16.63)
Mean body mass index (SD), Z-score	-0.68 (1.58)
Mean age (SD), years	11.3 (3.5)
Mean duration of diabetes mellitus (SD), years	3 (1.98)
Mean TSH (SD), mIU/L	3.76(8.48)

The normal limits for FT4 were 1.0-2.1 ng/dL in 2 to 7-year-olds and 0.8-1.9 ng/dL in 8 to 20-year-olds. The normal TSH limits were 0.7-5.7 mIU/L in 2 to 7-year-olds and 0.7-5.7 mIU/L in 8 to 20-year-olds. The diagnosis of primary hypothyroidism was made in those with low FT4 and high TSH. Subclinical hypothyroidism was diagnosed in those with high TSH and normal FT4; hyperthyroidism in those with low TSH and high T3 and T4; and subclinical hyperthyroidism in those with normal TSH and high T3 and T4.[12]

Results

There were 55 T1DM patients who regularly visited our OPC. Thirty-four patients met the inclusion criteria and were included in the study.

There were 2 male patients with high TSH levels, 25 and 45 mIU/L, respectively. These patients were subsequently found to have normal FT4 levels, hence, they were diagnosed with subclinical hypothyroidism. Mean TSH was 3.76 (SD 8.48) mIU/L, ranging from 0.033 to 45 mIU/L. Pearson's correlation test revealed no significant association between duration of diabetes and thyroid stimulating hormone concentration (rs=-0.068; P=0.703).

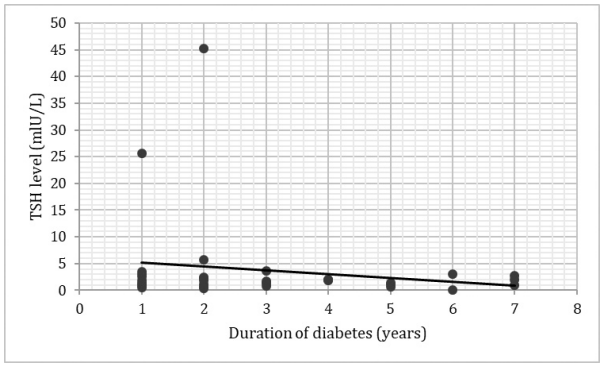

Figure 1. Association between duration of diabetes and TSH levels in children with T1DM

There were 2 patients with high TSH, one with duration of illness 1 year with TSH 25 mIU/L and one patient 2 years 45 mIU/L (**Figure 1**).

Discussion

The mean TSH concentration in our subjects was 3.76 (SD 8.48) mIU/L. *The International Society for Pediatrics and Adolescent Diabetes Mellitus* recommends that screening of thyroid function by measuring thyroid stimulating hormone (TSH) and anti-thyroid peroxidase antibodies at the time of diabetes diagnosis and, thereafter, every second year in asymptomatic individuals without goiter, or in the absence of thyroid autoantibodies. More frequent assessment is indicated otherwise.[13] Kabelitz *et al.* and Loviselli *et al.* reported the prevalence of autoimmune thyroiditis in population was 2.9-3.4%,[14,15] while Kalaoumenou *et al.* reported 4.6% in Greek population.[16] Subclinical hypothyroidism was found in 7-20%[5,7] compared to adults with rates of 1-10%,[7,8] and 2-6% in the general population of children.[9]

In our study, subjects' mean duration of diabetes was 3 years and mean age was 11.3 years, in which in the periode of puberty. Thyroid dysfunction apparent at diabetes onset[10,12] or years thereafter.[10,14,15]Peak of autoimmune thyroiditis incidence in the early until mid puberty.[17,18]

Two of our male patients were diagnosed with

subclinical hypothyroidism. Females have been reported to be at risk for autoimmune thyroiditis.[19,20] Sharifi *et al.* and Araujo *et al.* stated that gender predisposition of patients suffered from thyroid dysfunction was varied.[20,21] Subclinical hypothyroidism is frequently observed in T1DM.[13,21-27]

We found no significant association between duration of diabetes and TSH levels in children with T1DM. Past studies have shown that the longer the duration of diabetes, the higher the prevalence of autoimmune thyroiditis.[22--26] A previous study reported that prevalence of autoimmune thyroiditis in T1DM patients increased post- puberty.[25] Another study stated that the peak prevalence of thyroid antibody was observed after the age of 15 years or a duration of diabetes of 3.5 years.[26]

Thyroid stimulating hormone is a sensitive method to detect thyroid dysfunction. Normal TSH has a high negative predictive value to exclude thyroid disease and TSH changes can be detected earlier than FT4 changes. Ramasamy *et al.* stated that TSH >2.2 mIU/L was predictor of hypothyroidism in T1DM, with 83% sensitivity and 72% specificity.[28] However, TSH is of limited value for diagnosing hypothyroidism in central hypothyroidism and acute illness. The TSH needs to be rechecked after the acute illness to distinguish between non-thyroidal illness syndrome and actual hypothyroidism.[29] In addition, the TSH examination is less expensive than the thyroid antibody test. Screening once every 2 years is safe, effective, cost-efficient, as well as useful for avoiding the trauma of unnecessarily frequent blood sampling.[13]

In conclusion, there is no significant association between duration of diabetes and thyroid stimulating hormone in children with T1DM.

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Conflict of Interest

None declared.

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Original Article

Side effects of long-term antiepileptic drugs on renal tubules of Indonesian children

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Abstract

Background Long-term treatment with antiepileptic drugs such as valproic acid (VPA) and carbamazepine (CBZ) may disrupt renal tubular function. Urinary N-acetyl-beta-D-glucosaminidase (NAG) may reflect tubular function and may be useful in detecting early-stage tubular injury. To date, no study has investigated the toxic effect of VPA and CBZ on renal tubules using urinary NAG in Indonesian children.

Objectives To determine the toxicity of long-term VPA and/or CBZ treatment on renal tubules in children with epilepsy by measuring urinary NAG index (iNAG).

Methods This cross-sectional study was conducted from January to March 2015 at Cipto Mangunkusumo Hospital and Anakku Clinic Pondok Pinang, Jakarta. We included children aged 3 to 16 years with epilepsy on VPA (n=36), CBZ (n=14), or VPA-CBZ combination (n=14) therapy. We measured urinary levels of creatinine and NAG. The urinary NAG reference value was obtained from age-matched healthy controls (n=30). To eliminate diurnal variations in NAG, iNAG was calculated by dividing urinary NAG by urinary creatinine. A urinary iNAG of more than 2 standard deviations above the mean for healthy children was considered elevated.

Results Mean urinary iNAG values for the control, VPA, CBZ, and combination groups were 3.01, 5.9, 4.07, and 6.9 U/g, respectively. All treated groups had higher mean urinary iNAG values compared to the control group. Urinary iNAG was increased in 11/36 children on VPA, 2/14 children on CBZ, and 9/14 children on combination therapy.

Conclusion Long-term VPA use may impair renal tubular function, as shown by the increased urinary iNAG. Combination therapy increases damage in the renal tubules.**[Paediatr Indones. 2018;58:84-9; doi: http://dx.doi.org/10.14238/pi58.1.2018.84-9]**.

Keywords: *epilepsy; valproic acid; carbamazepine; urinary iNAG; renal tubular injury*

The kidneys perform essential functions of excretion and hormone production.[1] One of the excretory functions is to eliminate foreign chemicals (xenobiotics), such as drugs and their metabolites.[2] A long-term use of drug could be harmful for the kidney. Nephrotoxic agents account for approximately 20% cases of acute kidney injury (AKI).[3,4] The typical course of AKI involves multiple mechanisms including hemodynamic changes in the glomeruli, tubular cell toxicity, and interstitial nephritis. Despite the injury is usually preventable and reversible, the nephrotoxic agents should always be used cautiously.[5,6]

The glomerulus and renal tubules are responsible for excretory function. A number of different laboratory examinations are available to evaluate this function.[1] Serum creatinine is the most frequently used parameter to detect abnormalities of glomerular function; however, it is not specific and may not be helpful to detect signs of early AKI.[7] Although the decline of glomerular performance is due to the tubular injury, and vice versa, serum creatinine cannot

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be used to evaluate tubular function.[1] Therefore, there is an immediate need to find other biomarkers to detect kidney injury. Recently, studies have reported urinary N-acetyl-beta glucosaminidase (NAG) as a promising marker to detect the kidney's excretory function. The urinary level of NAG reflects the excretory performance of renal tubules, and increased urinary NAG indicates injury to the renal tubules.[8,9] Unfortunately, no universal reference value for normal urinary NAG values are currently available; previous studies have determined their own reference values according to the reagent or substrate used.

Epilepsy is a chronic clinical condition that requires long-term pharmacological treatment and may lead to a major morbidity. The incidence of epilepsy is approximately five to seven cases per 10,000 children per year. Epilepsy affects five in every 1,000 children aged zero to 15 years.[10,11] The high prevalence of epilepsy may result in the long-term use of antiepileptic drugs, such as valproic acid (VPA) and carbamazepine (CBZ). Over the last two decades, a number of studies have shown that VPA and CBZ are nephrotoxic. Although the incidence is unknown, previous reports have demonstrated that renal tubular injury in patients treated with VPA or CBZ is associated with high urinary NAG index (iNAG). This index calculates the ratio of NAG per urinary creatinine (U/g creatinine).[12-18] Since antiepileptic drug toxicity has a high interethnic variability,[19] it is necessary to determine this association in various ethnic groups.

The urinary iNAG of Indonesian children receiving antiepileptic drugs has not been previously documented. Observing the increase of urinary iNAG, instead of serum creatinine, will help clinicians to be recognize renal tubular injury at an earlier stage. This study aims to measure urinary iNAG as a marker of renal injury in Indonesian children with epilepsy who receive long-term treatment with VPA and CBZ.

Methods

This cross-sectional study was conducted from January to March 2015 at the Neurology Outpatient Clinic of the Department of Child Health, Cipto Mangunkusumo Hospital, and Anakku Clinic, Pondok Pinang, Jakarta, Indonesia. The subjects of this study were epileptic children who received valproic acid and carbamazepine as a single or combination therapy. Required subject number was calculated using single proportion formula, which determined that minimal 30 subjects was needed in each treatment groups. We included children who have received their antiepileptic drug regimen for at least 6 months who had no previous kidney or liver disease, no signs or symptoms of urinary tract infection, and normal serum ureum and creatinine levels. Patients with short stature or hypertension were excluded. The dose of the antiepileptic drugs was determined and adjusted as needed by the patient's treating pediatric neurologist. Sixty-four children aged three to 16 years were enrolled and assigned into three groups: VPA monotherapy (n=36), CBZ monotherapy (n=14), and VPA and CBZ combination therapy (n=14). Thirty clinically healthy and well-nourished children were selected consecutively from General Pediatric Clinic of Cipto Mangunkusumo Hospital, assigned as the age-matched control group.

We measured serum ureum and creatinine levels and performed urinalysis in all subjects. We also determined urinary creatinine and NAG levels. The measurement of urinary NAG level was done at Prodia Laboratories, Jakarta, by colorimetry using the 3-cresolsulfonphthaleinyl-N-acetyl-D-glucosaminide reagent (*Roche Diagnostics*, Indianapolis, USA). To eliminate diurnal variations in urinary NAG, iNAG was calculated by dividing the urinary NAG level by the urinary creatinine level. An eleveated iNAG was defined as an iNAG level higher than +2 standard deviations (SD) of the mean iNAG in the control group. The urine samples for iNAG measurement were kept in -20^{o}C temperature until all the samples were ready to be tested at the same time, to minimize bias associated with the reagent and assay tools.

The study protocol was approved by the Medical Research Ethics Committee of the Universitas Indonesia Medical School. We obtained informed assent and consent from each subject and his or her parent or guardian.

We used the unpaired T-test to evaluate the mean difference of iNAG between each individual treatment group and the control group. If the data did not meet the requirements for parametric testing, the Mann-Whitney test was employed. The differences in the proportion of subjects with elevated urinary

iNAG between the groups were analyzed using the Kruskal-Wallis test. P values of <0.05 were considered statistically significant.

Results

Subject characteristics are shown in **Table 1**. Mean (SD) urinary iNAG in the VPA, CBZ, VPA+CBZ, and control groups were 5.9 (4.17), 4.07 (2.36), 6.9 (2.98), and 3.01 (1.83) U/g creatinine, respectively (**Table 2**). The mean difference was statistically significant between the VPA and control groups (P=0.009) and between the VPA+CBZ and control groups (P<0.001). The difference in urinary iNAG between the CBZ and control groups did not reach statistical significance (P=0.244).

Using a cut-off level of +2SD of the control group mean (3.0+3.6 U/g creatinine), eleven out of 36 subjects in the VPA group, 2/14 subjects in the CBZ group, and 9/14 subjects in the VPA+CBZ group had elevated urinary iNAG. The difference between these three treatment groups was statistically significant (P=0.017) (**Table 3**).

Discussion

This study is the first in Indonesia to evaluate antiepileptic drug-induced renal tubular injury in children using urinary iNAG as a biomarker. Urinary iNAG is a more sensitive parameter of renal excretory function than serum ureum and creatinine to evaluate renal excretory function, since changes in the iNAG

Table 1. Subjects' characteristics

Characteristics	Group I VPA (n = 36)	Group II CBZ (n = 14)	Group III VPA+CBZ (n = 14)	Group IV Control (n = 30)
Median age (range), years	9 (4-14)	8.5 (4 - 14)	10 (3 - 16)	8 (3 - 14)
Gender				
Male	21	7	7	18
Female	15	7	7	12
Nutritional status				
Obese	14	4	3	5
Overweight	5	1	1	6
Well-nourished	15	8	8	17
Underweight	2	1	2	2
Length of therapy (range), months	16 (7-70)	28 (12-75)	20.5 (8-102)	-
Median dose of antiepileptic drugs (range), mg/kg/day				
VPA	20 (7.5-49)	-	30 (12.5-50)	-
CBZ	-	15 (10-30)	20 (10-45)	-

VPA: valproic acid; CBZ: carbamazepine

Table 2. The urinary iNAG levels in epileptic *vs.* healthy children

	VPA (n =36)	CBZ (n =14)	VPA+CBZ (n =14)	Control (n=30)
Mean iNAG (SD), U/g creatinine	5.919 (4.17)	4.07 (2.36)	6.9 (2.98)	3.01 (1.83)
P value	0.009	0.244	< 0.001	-

VPA: valproic acid; CBZ: carbamazepine; SD: standard deviation

Table 3. Subjects with elevated urinary iNAG

	VPA (n=36)	CBZ (n=14)	VPA+CBZ (n=14)	P value
Subjects with elevated iNAG	11	2	9	0.017

Cut-off level: + 2 SD control group [3.0 (SD 3.6) U/g creatinine group]

occur earlier than changes in serum ureum and creatinine levels. As iNAG is influenced by age, control subjects should be age-matched to their treated counterparts. We attempted to minimize measurement bias associated with the reagent and assay tools by performing the urinary NAG examination at the same time for all specimens.

We enrolled a total of 94 children, consisting of 53 boys and 41 girls. Twenty-six subjects were obese, 13 were overweight, 48 were well-nourished, and 7 were underweight. Obesity was most frequent in the VPA group, possibly due to a well-known side effect of the drug. Verotti *et al.*[20] reported higher weight gain in the first 3 months after consuming VPA is due to a multifactorial etiology. Several hypotheses explaining VPA-associated weight gain include hypothalamic dysregulation, hyperinsulinemia, insulin resistance, and genetic susceptibility.

The number of participants treated with CBZ did not meet the minimum sample requirement. Therefore, we expanded the age group from six to 12 to three to 16 years. However, the total number of subjects enrolled in the CBZ group and the VPA+CBZ group remained insufficient, at only 14 subjects in each group. The small sample size in those groups resulted in higher variability of urinary iNAG, as urinary iNAG level is influenced by age. The difficulty in recruiting subjects who received CBZ may be due to CBZ being less preferred by clinicians and patients due to its potential to cause the life-threatening side effect known as Stevens-Johnson Syndrome (SJS). Forty-percent of SJS cases are linked to CBZ, and its incidence is higher in Southeast Asians.[21-23]

We required our subjects to have taken anti-epileptic drugs for a minimum of six months, to anticipate the possibility of time dependency in the emergence of renal toxicity. In this study, the length of treatment across groups ranged from seven to 102 months. The monotherapy groups had relatively lower median doses of the respective drugs, while the combination therapy group had higher median doses for both VPA and CBZ, possibly reflecting epilepsies that are more difficult to manage. However, the median treatment duration in the VPA+CBZ group was not the highest amongst the three treatment groups, although the range was widest. This finding suggests that at least half of the patients in the combination therapy group were managed with more aggressive adjustments to their treatment regimens compared to their monotherapy counterparts.

Compared to the control group, iNAG was higher in the VPA monotherapy group and in the VPA+CBZ group. The highest iNAG value was observed in the VPA+CBZ group, indicating that renal tubular injuries are worsened by combination therapy. These findings are consistent with previous studies. Animal studies have shown that valproic acid blocks fatty acid β-oxidation in the mitochondria and stimulates peroxisome proliferation in the liver and kidney. In addition, valproic acid increases glutamine uptake and ammonia production in the renal tubules.[24] However, the mechanism occurring in the renal tubules remains unclear. In a cohort study by Verrotti *et al.*,[15] patients treated with VPA and CBZ had increased iNAG values after six months of therapy. Such a result was not observed in patients treated with phenobarbital. Unay *et al.*[17] similarly reported higher iNAG in children treated with VPA monotherapy, CBZ monotherapy, and combination therapy compared to untreated controls. However, there was no difference in iNAG between children receiving lamotrigine therapy and controls. This is supported by Mazaheri *et al.*,[18] who higher iNAG values in subjects using VPA and CBZ compared to healthy children and untreated epileptic patients. Three previous studies showed increased iNAG in patients receiving CBZ monotherapy compared to healthy controls.[15,17,18] In contrast, we found a slightly higher mean iNAG in the CBZ monotherapy group compared to the control group, but this difference was statistically insignificant. This could be due to the number of subjects in the CBZ group being fewer than the minimum required sample size.

Various methods are available to measure iNAG and its reference value. Consequently, it is difficult to compare and interpret iNAG from the different techniques reported. Csathy *et al.*[25] reported the first systematic review of various methods of iNAG measurement. This review compiled studies from 1962 to 1992 which applied various techniques, substrates/reagents, and different statistical analyses. Skalova and Chladek[26] evaluated iNAG values in 262 healthy children aged zero to eight years. They used the fluorimetry method, with creatinine expressed in units of nkat/mmol. The difficulty in converting urinary iNAG values measured using different methods and

reagents has necessitated each study to provide its own control group for reference values.

We also evaluated the proportion of subjects with increased iNAG based on a reference value of our control group mean + 2SD, yielding 3.0 (SD 3.6) U/g creatinine as our cut-off point. The proportion of subjects with elevated iNAG in the VPA+CBZ group was twofold that in the VPA group, and the proportion of subjects with elevated iNAG in the VPA group was in turn twice as high as in the CBZ group.

In a study by Korinthenberg *et al.*[12] involving 20 subjects receiving VPA, 27 subjects receiving CBZ, 9 subjects receiving ethosuximide, 8 subjects receiving phenobarbital, and 23 age-matched controls, increased iNAG was defined as the iNAG level higher than the 95th percentile of the control group iNAG values. In that study, 33% of subjects in the ethosuximide group, 20% of subjects in the VPA group, and 25% of subjects in the phenobarbital group had increased iNAG. The elevation of iNAG in the CBZ group was not statistically significant.

Similar to our study, Otsuka *et al.*[13] used +2SD urine iNAG of the control group as the cut-off point. The study by Otsuka also measured serum VPA level in subjects receiving VPA and found a serum VPA of >60 μg/mL in 47% of subjects and <60 μg/mL in 24% of subjects. In the same study, 38% subjects receiving CBZ and 25% subjects receiving combination therapy had elevated urine iNAG. Csathy *et al.*[16] used a similar cut-off point and found that 45% subjects receiving VPA, 26% subjects receiving CBZ, and 36% subjects receiving combination therapy had elevated urinary iNAG.

One of the limitations of our study was the inability to obtain age-specific baseline data on normal iNAG values due to the limited number of subjects in the control group. Therefore, we were only able to compare urinary iNAG values between each respective treatment group and the control group, and not between age groups. As we did not measure serum antiepileptic drug levels, we were unable to observe the association between the cumulative dose of the drug received and iNAG values. Moreover, our cross-sectional design did not allow the determination of the timing of the rise in urinary iNAG, so that this study is unable to inform clinicians on the optimal timing to evaluate the urinary iNAG in children receiving antiepileptic drugs.

To date, there is no recommendation on the further management of patients with elevated iNAG secondary to antiepileptic drug treatment. Nevertheless, clinicians should be more vigilant of the potential nephrotoxic effects of antiepileptic treatment, especially when prescribing combination therapy, as the consequences of elevated iNAG in subjects using VPA and CBZ can be observed only after long-term follow-up. These drugs should be given in an appropriate dose and duration to minimize the side effects.[12,18] Patients with increased iNAG should undergo further evaluation, dose adjustment, or, whenever possible, modification of therapy to favor non-nephrotoxic drugs, such as lamotrigine and levetiracetam. Such steps are important to prevent further impairment of kidney function. These patients should be closely monitored and their parents should be informed of the potential side effects of the drugs received, any alternative therapeutic regimens available, and further examinations needed. Effective communication between parents, patients, and clinicians will improve the outcomes of patients who already show increased iNAG due to antiepileptic drug treatment.

In conclusion, the mean iNAG in children with epilepsy receiving VPA is twice as high as that in healthy children. In children receiving VPA and CBZ combination, the mean iNAG is 2.3 times higher than in healthy children. Children receiving VPA and CBZ combination therapy have the highest proportion of elevated iNAG, followed by those receiving VPA monotherapy. Urinary iNAG should be monitored in children receiving VPA and CBZ to detect drug-induced nephrotoxicity. A prospective study involving periodical measurement of urinary iNAG is essential to help clinicians determine the ideal timing of urinary iNAG monitoring.

Conflict of Interest

None declared.

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Original Article

Association between oxygen saturation and critical congenital heart disease in newborns

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Abstract

Background Critical congenital heart disease (CCHD) is relatively common, with a prevalence of 6-8 in every 1,000 live births. This congenital anomaly is a newborn condition that would be ideally suited for a screening program, if simple and reliable methods were available. Pulse oximetry (PO) has been proposed as a screening method to detect CCHD.

Objective To assess for a possible association between decreased oxygen saturation and CCHD in newborns.

Methods We conducted a cross-sectional study from March 2014 to February 2015 in several hospitals in North Sumatra. Healthy, full term and post-term newborns aged 2 to 72 hours underwent pulse oximetry measurements on the right hand and one of the lower extremities. If oxygen saturation (SpO2) was ≤ 95%, the measurement was repeated 2 more times. Subjects also underwent echocardiography.

Results A total of 386 newborns underwent SpO_2 measurements: 377 newborns had SpO_2 > 95% and 9 newborns had SpO_2 ≤ 95%. Of the infants with SpO_2 > 95%, 297 were excluded because their parents refused echocardiography examination. Thus, 80 newborns with SpO_2 > 95% and 9 newborns with SpO_2 ≤ 95% underwent echocardiography. Echocardiography revealed that 5 of 9 newborns with SpO_2 ≤ 95% suffered from Tetralogy of Fallot (ToF) (3 subjects) and transposition of the great arteries (TGA) (2 subjects). One infant with SpO_2 > 95% had ventricular septal defect (VSD), as detected by echocardiography. Oxygen saturation ≤ 95% had significant association with CCHD (P<0.001).

Conclusion Decreased oxygen saturation has a significant association with critical congenital heart disease in newborns. **[Paediatr Indones. 2018;58:90-4; doi: http://dx.doi.org/10.14238/pi58.1.2018.90-4]**.

Keywords: *: pulse oximetry; oxygen saturation; critical congenital heart disease; newborn*

Congenital heart disease (CHD) is the most common group of significant congenital abnormalities. It may present with an asymptomatic murmur detected on routine neonatal examination.[1] Among all congenital malformations, cardiac lesions are the most common, with a prevalence of approximately 6 to 8 per 1,000 live births. Early diagnosis of CHD is important because delayed treatment of severe CHD can lead to cardiac failure, cardiovascular collapse, and even death. However, such early diagnosis of CHD in the first few days of life is difficult.[2]

Congenital heart disease accounts for about 10% of infant deaths due to congenital malformations.[3] Early detection of ductal-dependent CHD is important in newborns prior to ductal closure,[2] thus, screening for these abnormalities in newborns should be done prior to hospital discharge. For this purpose, pulse

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oximetry (PO) can be a sensitive method to detect these abnormalities.[4]

Pulse oximetry has been proposed as one such strategy to be an effective, noninvasive, and inexpensive tool, allowing earlier diagnosis of CCHD.[5,6] Screening with PO has been suggested as a useful strategy for detecting defects with decreased arterial oxygen saturation (SpO_2), before heart failure and circulatory collapse develops.[7] Several studies have documented the lack of sensitivity of routine neonatal examination in detecting CHD. The aim of this study was to assess for a possible association between decreased oxygen saturation and detecting CCHD in newborns.

Methods

This cross-sectional study was conducted from March 2014 to February 2015 in Medan, North Sumatera, Indonesia in four hospitals, one maternity and three general hospitals. All newborn infants >36 weeks gestational age and birth weight ≥2,500 grams born during the one-year study period underwent pre-ductal (finger probe) and post-ductal (foot probe) PO measurements. Premature infants below 36 weeks gestation and infants with respiratory disorders were excluded from the study. Subjects were selected by consecutive sampling. Pulse oximetry was performed on asymptomatic newborns using a *Tuffsa*t pulse oximeter (GE Ohmeda, Finlandia) with a reusable probe.

Measurements were performed by doctors and nurses at the Post-natal Ward on infants aged 2 to 72 hours, on either the right or left foot while the infant was quiet. Infants with $SpO_2 \leq 95\%$ on the lower extremities had the readings repeated twice after 1 hour. Infants whose SpO_2 remained ≤ 95% after the repeated measurement underwent echocardiography.

The study was approved by the Research Ethics Committee of the University of Sumatera Utara Medical School. Informed consent was obtained from subjects' parents. Basic characteristics of subjects were obtained from interviews, questionnaires, and physical examinations. Gestational age was assessed using the new Ballard score.[8] Birth weight was categorized as between 2,500 to 2,999g, 3,000 to 3,499g, 3500 to 3999 g, and >4,000g. Infant age at the time of screening was categorized as <24 hours, 24 to 36 hours, 37 to 48 hours, and >48 hours.

Analyses were performed with *SPSS statistical software version 17.0*. The association between oxygen saturation and CCHD was analyzed by Chi-square test. P values <0.05 were considered to be statistically significant.

Results

Our study subjects were predominantly male (49 newborns). The majority of subjects had birth weights in the 3,000g to 3,499g category (58 newborns), and were 37 to 48 hours of age (**Table 1**).

During the study period, 386 newborns underwent PO measurements. Of these, 297 infants were excluded because their parents refused echocardiography examination. The remaining 89 newborns underwent echocardiography, 80 of whom had SpO_2>95% and 9 of whom had $SpO_2 \leq 95\%$. Echocardiography revealed that 5 of 9 newborns suffered from either ToF (3 subjects) or TGA (2 subjects). One infant with SpO_2>95% had a VSD, detected on echocardiography. Oxygen saturation ≤ 95% had a significant association with CCHD (P<0.001).

Table 1. Demographic characteristics of subjects

Subject characteristics	(N = 89)
Sex, n	
Male	49
Female	40
Age, n	
< 24 h	18
24-36 h	28
37-48 h	34
> 48 h	9
Birth weight, n	
2,500 – 2,999 g	13
3,000 – 3,499 g	58
3,500 – 3,999 g	17
≥ 4,000 g	1

Table 2 shows the echocardiographic findings in our 89 subjects. In infants with cyanotic CHD (SpO2 ≤ 95%), 3 newborns had ToF and 2 newborns had TGA. In newborns with SpO2 > 95%, 1 infant had a VSD.

Table 2. Echocardiography findings after SpO_2 measurement

	Age, hours	Pre-ductal/ post-ductal $SpO_2 \leq$ 95%	Pre-ductal/ post-ductal $SpO_2 >$ 95%
TGA	7-22	71-80 / 65-84 (n=2)	None
ToF	5-26	88-89 / 86-90 (n=3)	None
VSD	28	None	98/96 (n=1)
Normal	<24 - >48	87-94 / 89-92 (n=4)	96-100 / 96-100 (n=79)

ToF=tetralogy of Fallot, TGA=transposition of the great arteries, VSD=ventricular septal defect

Of the 9 infants with SpO2 ≤95%, five were diagnosed with CCHD by echocardiography, while 4 infants had normal echocardiograms. None of the subjects with SpO2 > 95% had VSD, as evaluated by echocardiography. Chi-square analysis revealed a significant correlation between ≤ 95% oxygen saturation and CCHD in newborns (P=0.001) (**Table 3**).

Table 3. Association between CCHD and SpO_2 values

	CCHD	Normal	Total	P value
$SpO_2 \leq$ 95%	5	4	9	0.001
$SpO_2 >$ 95%	1	79	80	
Total	6	83	89	

Discussion

This study was conducted from March 2014 to February 2015 in several hospitals in Medan and Lubuk Pakam: H. Adam Malik Hospital, Stella Maris Hospital, Sundari Hospital, and Grand Medistra Hospital. Of 89 newborns who underwent screening for SpO_2 followed by echocardiography, 9 had SpO_2 <95%, while the rest had SpO_2 > 95%.

Congenital heart diseases are the most common group of congenital malformations and a leading cause of infant death in developing countries.[9] The clinical manifestations of this disorder vary from mild to severe. In the mild form, patients may have no symptoms or abnormalities on clinical examination. However, those with severe CHD may have clear symptoms since birth that require emergency procedures. Globally, congenital heart disease affects more than one million of live births per year, due to structural cardiac and major blood vessels disorders appearing shortly after birth.[10]

Early detection in asymptomatic newborns can prevent severe consequences of illness caused by delayed diagnosis.[11] Screening of asymptomatic newborns with PO is a promising strategy for early detection of critical congenital heart disease (CCHD), because it manifests only as a decrease in SpO_2.[12] According to the *American Academy of Pediatrics* (AAP) and *American Heart Association* (AHA), seven lesions were targeted for screening by PO: truncus arteriosus, TGA, tricuspid atresia, ToF, total anomalous pulmonary venous return (TAPVR), hypoplastic left heart syndrome (HLHS), and pulmonary atresia.[9] Pulse oximetry screening for duct-dependent CHD in newborns is important to perform before hospital discharge, as pulse oximetry is sensitive enough to detect these abnormalities.[4]

Oxygen saturation generally reaches 95% within 1 hour after birth. Cyanotic CHD babies usually have oxygen saturation of less than 88%.[11] We assessed for an association between low oxygen saturation and CCHD in newborns within 2 to 72 hours after birth, and before hospital discharge. A previous study in California also conducted screening in 13,287 newborns aged 21 hours to 36 hours after birth.[13]

Our study showed that low oxygen saturation occurs in newborns with ToF, with 88% to 89% pre-ductal and 86% to 90% post-ductal SpO_2. For TGA, oxygen saturations were 71% to 80% pre-ductal and 65% to 84% post-ductal (**Table 2**). These findings were similar to those of previous studies in the UK and Sweden that screened newborns' oxygen saturation to attempt to identify CCHD.[8,14] Another study also showed high sensitivity of pulse oximetry in 75% of critical lesions and 49% of other types of CHD in all asymtomatic newborns.[14]

Chi-square test was used to analyze for an association between decreased SpO_2 and CCHD. We found that 5 of 9 infants with $SpO_2 \leq$ 95% had cyanotic congenital heart disease by echocardiography. As such, $SpO_2 \leq$ 95% had a significant association with CCHD (P=0.001) (**Table 3**). We noted that 79 of the 80 newborns who had SpO_2>95% showed normal echocardiograms. Similarly, an Indian study found that

of 1,200 newborns screened using pulse oximetry with 66.6% sensitivity and specificity 99.90%, there were 6 with SpO2 ≤ 95%. These 6 children underwent echocardiography, which showed one with TGA, two with truncus arteriosus, and three with normal echocardiography.[15] In addition, a Chinese study found that 46 of 49 newborns (94%) born without symptoms suffered from congenital heart disease. Also, in 8 of 8 newborns (100%) born without symptoms, critical congenital heart disease could be detected by pulse oximetry screening and physical examination when the baby was discharged.[16]

In our study, one newborn had SpO_2 > 95% and echocardiography revealed the child to have acyanotic CHD. Also, a previous study in Sweden found 10 newborns with SpO_2 > 95% screening results, but echocardiography showed one child to have a CHD.[14]

Limitations of this study were the small sample size and not assessing the sensitivity and specificity of the pulse oximetry. Further study is needed with a larger sample size to assess the prevalence and incidence of CHD in North Sumatra. An advantage of this study was that it is a pilot study to assess for an association between oxygen saturation and cyanotic congenital heart disease in newborns, whereas previous studies only assessed the effectiveness and accuracy of pulse oximetry to detect critical congenital heart disease in newborns. In conclusion, decreased oxygen saturation has a significant association with critical congenital heart disease in newborns.

Conflict of Interest

None declared.

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Original Article

Risk factors and the occurrence of cerebral palsy in high risk infants

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Abstract

Background The incidence of cerebral palsy (CP) has increased due to better survival rates of high-risk babies. Early detection and time to the occurrence of CP in the first year of life is important in order to provide early intervention.

Objectives To determine the proportion of CP in high-risk babies, the time to the occurrence of CP in the first year, and assess possible associations between risk factors of CP and time to the occurrence of CP.

Methods A prospective cohort study was done on 150 high-risk babies up to the age of 12 months. We obtained history of motor ability and assessed primitive reflexes and postural reactions of subjects at the ages of 4 and 6 months. The diagnosis of CP was established at 6 and 12 months of age.

Results The proportion of CP was 26% at 6 months and 24% at 12 months of age. Significant risk factors associated with CP at 6 and 12 months of age were cerebral ultrasound abnormalities, hypoxic-ischemic encephalopathy, and intracranial hemorrhage. In 88.7% of subjects with CP, CP was detected in the first 6 months. Mean age at the occurrence of CP was 9.99 months (95%CI 9.46 to 10.53). Risk factors that significantly affected the time to the occurrence of CP by survival analysis were ultrasound abnormalities and hypoxic-ischemic encephalopathy.

Conclusions Cerebral palsy can be detected as early as the first 6 months of life. Cerebral ultrasound abnormalities and hypoxic ischemic encephalopathy are the risk factors associated with CP.

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Keywords: *early detection; cerebral palsy; proportion; risk factors; time to the occurrence of CP*

The incidence of cerebral palsy (CP) is 1.2 to 2.5 per 1,000 live births. Several factors, including prematurity, influence the occurrence of CP.[1] In Canada, the mortality of premature infants has declined from 256 per 1,000 live births in 1993 to 114 per 1,000 live births in 2002, accompanied by a rise in the rate of CP from 44.4 to 100 cases per 1,000 live births in the same period.[2] A similar trend has been observed in Sweden and Western Australia.[1]

Cerebral palsy is a static, non-progressive disorder of motor and postural function due to an insult on the developing brain, which results in motor delays as well as postural and motion abnormalities.[1] Some children with CP acquire various comorbidities and complications which may pose health threats and influence their quality of life.[3] Early detection of CP within the first year of life is essential to enable early intervention, which will affect the natural course of the disease.[4]

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Prematurity and low birth weight are risk factors for CP.[1,2] Theoretically, meningitis, intracranial hemorrhage (IC), and hypoxic ischemic encephalopathy (HIE) are also risk factors for CP due to brain injury.[4] Survival analysis on the time to the occurrence of CP in high-risk babies has yet to be established, despite the importance for early prediction of CP in high-risk babies. In Indonesia, the higher survival rate of premature and other high-risk babies has also led to an increase of CP cases. High-risk babies are at risk of developing CP at a later age, due to risk factors occurring in the pre-, peri-, and post-natal periods.

This study aimed to determine the proportion of CP in high-risk babies, the time to the occurrence of CP in the first year, as well as risk factors as they pertain to the time to the occurrence of CP.

Methods

The main design of this study was prospective. We followed a cohort of high-risk babies to the age of 12 months. The study was done in Cipto Mangunkusumo Hospital, Jakarta, from April 2010 to July 2012. During the follow-up period, we performed bi-monthly assessments comprising of motor development history and clinical-neurological examinations. A survival analysis was done using data obtained at each of these bi-monthly assessments, with the occurrence of CP as the endpoint.

Using the appropriate formula, the minimum number of subjects required was 180. Inclusion criteria were high-risk babies, as signified by prematurity (gestational age of ≤ 32 weeks), low birth weight (birth weight < 2,499 g) and very low birth weight (birth weight of ≤ 1,500 grams), full term or preterm neonates with meningitis, moderate or severe HIE, ICH, and >48 hours of mechanical ventilation. We excluded infants with central nervous system malformations, genetic, chromosomal, or metabolic anomalies, neuromuscular disorders, or congenital infections. The independent variables were (1) risk factors; (2) cerebral ultrasound results; (3) motor delays; (4) primitive reflexes (palmar grasp, fisting, withdrawal, crossed-extensor, and traction response); and (5) postural reactions (protective-extension reflex and parachute reaction). The dependent variable was the occurrence of CP as determined by the gold standard examination of muscle tone and increased physiological reflexes at the specified age.

When subjects were 4 to 5 months of age, we performed the first motor development assessment and neurological examination comprising withdrawal reflex, palmar reflex, traction response, fisting, and crossed extensor reflex. At 6 months, motor development was again assessed, as well as all neurological examination items previously evaluated, with the addition of protective extension reflex. At 9 to 10 months, we again followed up the subjects' motor development and performed all neurological examination items evaluated previously, with the addition of parachute reaction. The presence of CP was officially determined at the ages of 6 and 12 months. We use the term 'officially' here so as to clarify that previous bi-monthly assessements were also done, as seen in the survival analysis in **Figure 1**. The diagnosis of CP was made by one of two experienced pediatric neurologists when abnormalities in muscle tone and increased physiological reflexes were found, without evidence of regression or progression.

Using assessment of CP based on clinical manifestation at 6, and 12 months of age, we determined the proportion of CP in high-risk babies at 6 and 12 months of age and determined the association between risk factors and CP. We used Kaplan-Meier survival analysis for the time of occurrence of CP in the first year of life, and the contribution of each risk factor. Significant risk factors were then subjected to multivariate Cox regression analysis. The study protocol was approved by the Medical Research Ethics Committee of the University of Indonesia.

Results

During the study period, 178 high-risk babies underwent screening for possible inclusion to the study. Out of these, 150 fulfilled the criteria for cohort analysis; 28 subjects were excluded (14 died and 14 were lost to follow-up due to undocumented address changes). At 6 months of age, 39/150 subjects (26%) had CP, and at 12 months of age 36/150 subjects (24%) had CP. For Kaplan Meiyer 14 died subjects have been participated for analysis. Subjects' characteristics are shown in **Table 1**.

On bivariate analysis, risk factors found to be associated with CP at the ages of 6 and 12 months

Table 1. Subjects' characteristics

Characteristics	(N=150)
Sex, n (%)	
Male	65 (43)
Female	85 (57)
Gestational age, n (%)	
≤32 weeks	120 (80)
>32 weeks	30 (20)
Birth weight, n (%)	
≤1,500 g	113 (75)
>1,500 g	37 (25)
Meningitis, n (%)	
Yes	5 (3)
No	145 (97)
Intracranial hemorrhage, n (%)	
Yes	19 (13)
No	131 (87)
Hypoxic-ischemic encephalopathy, n (%)	
Yes	7 (5)
No	143 (95)
Mechanical ventilation, n (%)	
Yes	30 (20)
No	120 (80)
Duration of mechanical ventilation, n (%)	
≥48 hours	24 (16)
<48 hours	6 (4)
Cerebral ultrasound, n (%)	
Abnormal	35 (23)
Normal	115 (77)

were cerebral ultrasound abnormalities, HIE, and ICH (**Table 2**). Sex, birth weight, meningitis, and duration of mechanical ventilation were not significantly associated with CP. Gestatational age was risk factor for CP at 12 months of age, but not at 6 months of age.

We performed a survival analysis on all subjects to determine the time of occurrence of CP during the 12 months of follow-up, as well as associated risk factors. Censored was a subject who has undergone effect (CP or died). The cumulative proportion surviving (CPS) was the sum of subjects without CP. **Figure 1** shows that the CPS at 6 months of age was 74% [standard error (SE) 3.5%], whereas CPS at 12 months of age was 70.7% (SE 3.7%). Mean age at the occurrence of CP was 9.99 months (95%CI 9.46 to 10.53).

Table 3 shows the survival analysis of the time to the occurrence of CP, based on risk factors. On bivariate analysis, factors significantly associated with survival, i.e., the time to the occurrence of CP, were gestational age of ≤32 weeks, cerebral ultrasound abnormalities, ICH, HIE, and meningitis. Cox regression analysis revealed that cerebral ultrasound abnormalities and HIE were significant risk factors for the occurrence of CP (**Table 4**).

Table 2. Bivariate association between risk factors and cerebral palsy at 6 and 12 months

Variables	CP at 6 months			CP at 12 months		
	OR	95%CI	P value	OR	95%CI	P value
Sex	1.04	0.60 to 1.82	0.87	1.22	0.68 to 2.17	0.5
Male	(reference)			(reference)		
Female						
Gestational age	0.64	0.36 to 1.13	0.14	0.5	0.29 to 0.88	0.022
≤32 weeks	(reference)			(reference)		
>32 weeks						
Birth weight	1.8	0.82 to 3.95	0.12	1.64	0.74 to 3.62	0.20
≤1500 g	(reference)			(reference)		
>1500 g						
Meningitis	0.76	0.13 to 4.49	0.76	0.83	0.14 to 4.89	0.83
Yes	(reference)			(reference)		
No						
Intracranial hemorrhage	4.31	2.8 to 6.6	<0.001	4.49	2.75 to 6.99	
Yes	(reference)			(reference)		<0.001
No						
Hypoxic-ischemic encephalopathy	4.47	3.29 to 6.1	<0.001	4.91	3.56 to 6.82	
Yes	(reference)			(reference)		<0.001
No						
Mechanical ventilation			0.85			0.84
>48 hours	1.12	0.32 to 3.9		1.12	0.32 to 3.92	
<48 hours	(reference)			(reference)		
Cerebral ultrasound	10.95	5.77 to 20.8	< 0.001	13.6	6.54 to 28.35	<0.001
Abnormal	(reference)			(reference)		
Normal						

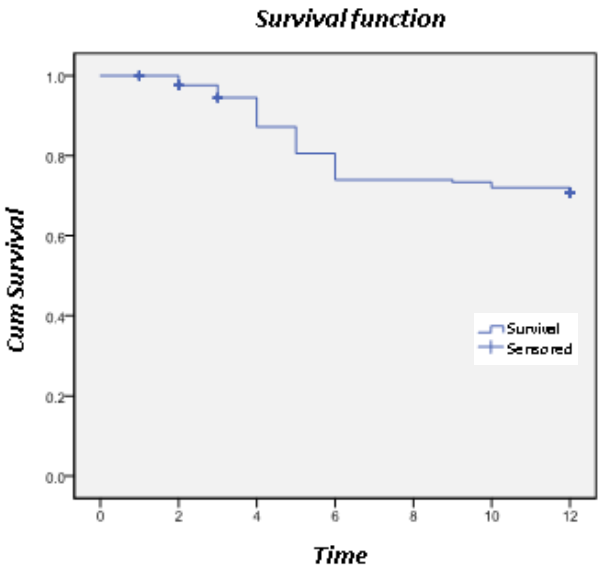

Figure 1. Survival analysis of the time to the occurrence of CP

Discussion

The limitations of this study were the recruitment of subjects in a tertiary referral hospital, possibly leading to a higher proportion of CP than would be found in the general population, and follow-up largely done by home visits by the principal investigator only. However, this study has the advantage of being the first to describe survival risk based on the time of occurrence of CP in the first year of life in high-risk babies, as well as differential survival based on risk factors.

The proportions of CP in our subjects were 26% at 6 months of age and 24% at 12 months of age. Similarly, Zafeiriou *et al.* obtained an incidence of 28.5% in 204 high-risk babies.[5] The difference between the incidence at 6 and 12 months of age may be explained by the normalization of neurological

Table 3. The time to the occurrence of CP based on risk factors

Variables	CPS, %	SE, %	Mean time to CP, months	95%CI	Log-rank P value
Gestational age					
≤32 weeks	73.7	4	10.27	9.71 to 10.82	0.047
>32 weeks	58.1	6.5	8.92	7.46 to 10.38	
Birth weight					
≤1500 grams	68.2	4.4	9.92	9.31 to 10.53	0.42
>1500 grams	78.3	6.5	10.24	9.17 to 11.31	
Meningitis					
Present	50	20.4	8.95	5.24 to 11.76	0.041
Absent	71.5	3.7	10.39	9.5 to 10.59	
ICH					
Present	60.5	7.5	8.95	7.81 to 10.09	0.052
Absent	74.7	4.1	10.39	9.82 to 10.97	
HIE					
Present	0	0	3.35	2.64 to 4.05	<0.001
Absent	74.4	3.61	10.35	9.85 to 10.85	
Mechanical ventilation					
>48 hours	61.6	9.7	9.14	7.62 to 10.67	0.932
≤48 hours	66.7	19.2	8.83	5.24 to 12.42	
Cerebral ultrasound					
Abnormal	11	5.2	5.44	4.51 to 6.36	<0.001
Normal	89.6	2.9	11.4	11.07 to 11.79	

CPS: cumulative proportion surviving without CP; SE=standard error; ICH=intracranial hemorrhage; HIE= hypoxic-ischemic encephalopathy

Table 4. Risk factors significantly associated with the time to the occurrence of CP

Variables	β	SE	Wald	df	Sign	Exp(β)	95%CI
Cerabral ultrasound	2.799	0.380	54.206	1	0.000	16.421	7.796 to 34.590
HIE	1.332	0.475	7.852	1	0.005	3.785	1.492 to 9.620
Mechanical ventilation	0.057	0.389	0.021	1	0.884	1.085	0.494 to 2.268
ICH	-0.071	0.042	0.042	1	0.838	0.932	0.473 to 1,834
Prematurity	-0.488	0.396	1.516	1	0.218	0.614	0.282 to 1,335
Meningitis	1.298	0.635	4.176	1	0.041	3.662	1.054 to 12,717

SE=standard error; HIE= hypoxic-ischemic encephalopathy;ICH=intracranial hemorrhage;

features over time, possibly due to intervention or CNS maturation, or by the worsening of such features over time. Our results support the notion that clinical manifestations of CP can change with increasing age, particularly in the first year of life.[6]

We did not find a significant birth weight or gestational age differences in the incidence of CP. In contrast, other studies stated that prematurity and low birth weight were risk factors of CP.[1,2] This finding may be due to improved perinatal health services and medical technology, enabling better hemodynamic monitoring leading to prevention of extreme fluctuations of cerebral blood flow, thus reducing the rate of complications such as ICH in infants born with a birth weight of 1,000-1,500 grams and infants born at 28-32 weeks' gestational age.[7] Only 30/150 subjects (20%) needed mechanical ventilation. Cools *et al.* reported that 90% of infants born at <30 weeks' gestation required mechanical ventilation.[8] This difference may be caused by the difference in gestational age in the inclusion criteria, or due to advances in the management of premature babies, including surfactant therapy and the use of continuous positive airway pressure (CPAP), thereby reducing the need for mechanical ventilation.[9]

Cerebral ultrasound abnormalities were found in 35 subjects (23.3%). Six out of these 35 subjects developed CP. There was a significant difference in the proportion of CP in infants with abnormal ultrasound results compared to those with normal ultrasound results (P<0.001). This result concurred with previous reports that ultrasound abnormalities, especially grade 3 and 4 intraventricular hemorrhage (IVH), PVL, and ventriculomegaly are associated with CP or other abnormalities of motor development.[10-12] All subjects with moderate or severe HIE (n=7) had CP, a significant difference from the proportion of CP in subjects with no or mild HIE (P< 0.001). Our result was in agreement with previous studies that reported HIE, particularly in term infants, causing tissue damage in the form of PVL, focal and multifocal ischemia, and cerebral tissue necrosis.[13,14] Full term infants made up the majority of the infants with HIE in this study (5/7). Forty-three out of 150 subjects (28.6%) had ICH; 39.5% of these had CP. There was a significant difference in CP incidence in the ICH group compared to the non-ICH group, possibly due to the large proportion of grade 3 and 4 IVH found in the ICH group, which potentially develops into PVL cysts.[15]

On bivariate analysis, HIE, ICH, and ultrasound abnormalities showed significant associationS with CP (P<0.001) at 6 and 12 months. Moderate and severe HIE were significant risk factors of CP, as were grade 3 and 4 IVH. Ultrasound abnormalities associated with CP include PVL, grade 3 and 4 IVH, encephalomalacia, meningitis, hydrocephalus, and ventriculomegaly. Our results agree with current literature.[10-12]

We performed a survival analysis to determine the time to the occurrence of CP. Most subjects who had CP were diagnosed by the age of 6 months. Our findings suggest that the first 6 months is an important window for clinicians and parents to closely observe infants for signs of CP to enable early intervention for better outcomes. Multivariate Cox regression analysis showed that only cerebral ultrasound abnormalities, HIE, and meningitis significantly affected occurrence of CP.

In conclusion, the proportions of CP in our subjects are 26% at 6 months and 24% at 12 months. In 88.7% of subjects, CP is detected in the first 6 months. Significant risk factors related to the occurrence of CP and survival analysis are cerebral ultrasound abnormalities, hypoxic-ischemic encephalopathy, and intracranial hemorrhage.

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Conflict of interest

None declared

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Original Article

Correlation between immunization status and pediatric diphtheria patients outcomes in the Sampang District, 2011-2015

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Abstract

Background The number of diphtheria cases recently increased, such that an outbreak was declared in East Java Province, which includes the Sampang District. Immunization completion status is a determining factor for diptheria infection.

Objective To investigate for correlations between immunization status and outcomes (severity level, fatality, and complications) of diphtheria patients in the Sampang District.

Methods This analytic, cross-sectional study used secondary data from the East Java Provincial Health Office on diphteria patients aged 0-20 years during the 2011-2015 outbreak in the Sampang District and interviews with diphtheria patients in that region. *The Diphtheria Research Team* of Dr. Soetomo Hospital collected data on immunization status, diphtheria severity (mild, moderate, or severe), case fatality (died or survived), and complications in the patients (with or without complications). Spearman's, Chi-square, and Fisher's exact tests were used for data analyses, accordingly.

Results Seventy-one patients with clinical diphtheria were identified, 17 of whom were confirmed with positive culture results. The case fatality rates were 7% in patients with clinical and 5.9% in confirmed diphtheria. There were no correlations between patient immunization status and severity (P=0.469 clinical, P=0.610 confirmed), or fatality (P=0.618 clinical, P=0.294 confirmed) of diphtheria in the clinical and confirmed diphtheria patients. However, there was a correlation between patient immunization status and the emergence of complications in clinical (P=0.013), but not in confirmed (P=0.620) diphtheria patients.

Conclusion There is a correlation between immunization status and complications in clinical diphtheria patients. Such a correlation is not found in confirmed diphtheria cases because none of the patients had complete immunization status. **[Paediatr Indones. 2018;58:110-15; doi: http://dx.doi.org/10.14238/pi58.3.2018.110-15]**.

Keywords: *diphtheria; immunization status; diphtheria severity; fatality; complication*

Diphtheria is an acute infectious disease that mainly affects infants and children in the early years of life.[1] The number of diphtheria cases has decreased throughout the world. However, the East Java Provincial Government of Indonesia declared a diphtheria outbreak due to a high number of diphtheria cases in the province, including the Sampang District. From 2009 to 2012, there were approximately 1,870 reported diphtheria cases in the province, with 643 cases clinically diagnosed with diphtheria in healthcare centers. Out of 643 cases of diphtheria based on calinical diagnosis, fifty of them were confirmed by positive culture results based on official reports released between 2013 to 2014.[2,3] In the Sampang District alone, approximately 91 cases were found from 2011 to 2015.

Diphtheria is caused by the Corynebacterium diphtheria bacterial exotoxin.[1] The diphtheria toxin's main mode of action is to inhibit protein synthesis. This toxin may spread relatively quickly to different parts of

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the body, causing complications in the patients, such as myocarditis and neurologic dysfunction.[4-6] Diphtheria is transmitted mainly by droplets from sneezing and coughing, but also may be transmitted through direct contact with cutaneous diphtheria wounds.[7] Initially, the signs and symptoms of diphtheria are non-specific, such as malaise, fever around 38° Celsius, hoarseness, sore throat, and rhinorrhea. As the disease progresses, pseudomembranes develop in most cases, which may cause respiratory tract obstruction. This obstruction may lead to patient fatality.[7] A definitive diagnosis of diphtheria is carried out by obtaining specimens from suspected lesions and culturing them in media specific for Corynebacterium diphtheria.[4,6,7] Supportive examinations may also be carried out to assist the diagnostic process, in form of polymerase chain reaction (PCR), antibiotic-sensitivity test to identify antimicrobial resistance, Elek test to identify diphtheria toxin, and Shick test to identify serum antibodies against the diphtheria toxin.[8,9]

The number of diphtheria cases has been significantly reduced since the discovery and introduction of a diphtheria vaccination. Vaccine administration stimulates the production of antibodies against diphtheria toxin, mainly IgG and IgA, making the toxin unable to bind to the toxin receptor during future infection.[4] Studies have shown that of many factors affecting diphtheria spread in society, immunizations stand out as the most significant and influential factor.[10] We aimed to identify correlations between immunization status and diphtheria severity level, fatality, and complications in the Sampang District during the diphtheria outbreak from 2011 to 2015.

Methods

This study was an analytic study with a cross-sectional approach. Subjects were patients with clinical diagnoses of diphtheria in the Sampang District of Madura Island, East Java. Data were obtained from the East Java Provincial Health Office on diphtheria patients from 2011-2015 and by interview conducted towards the patient of the 2011-2015 diphtheria outbreak in the region by the Diphtheria Research Team of Soetomo Hospital consisting of pediatrician, residents, doctors, epidemiologists, public health officers, nurses, and medical students. The inclusion criteria were patients with a clinical diagnosis of diphtheria, maximum age of 20 years at the time of diagnosis, complete data and/or medical records, and diagnosed as not immunocompromised by a medical doctor. The minimum required number of subjects was calculated using the hypothesis test formula for two samples, and found to be 56.

The independent variable in this study was the patient immunization status, which was classified as "complete," "incomplete," or "unimmunized," according to the Indonesian Ministry of Health standards.[11] The dependent variables were the degree of severity, fatality, and complications in the patients. The degree of diphtheria severity was classified according to the *2008 Diagnosis and Therapy Guidelines* of Dr. Soetomo General Hospital, Surabaya, Indonesia, into "severe," "moderate," or "mild" diphtheria. Fatality was defined as the final outcome of of death due to diphtheria. Complication was defined as the presence of bullneck, myocarditis, shock, or bleeding manifestation.[6] Spearman's, Chi-square, and Fisher's exact tests were used for statistical analyses by *SPSS Statistics 23.0* software. This study was approved by the Ethics Committee for Health Research of Airlangga University Medical School, Surabaya.

Results

There were 81 diphtheria patients recorded in the Sampang District during 2011-2015. Ten patients were not included because of aged ≥ 20 years and/or incomplete data. Seventy-one patients with clinical diagnoses of diphtheria were included in this study, 17 (23.9%) of whom had positive culture results for Corynebacterium diphtheriae. Most of them were positive for *mitis biovar,* as described in **Table 1**.

Most diphtheria patients were female and characteristics of the patients were relatively similar between the two groups (clinical and confirmed

Table 1. Culture results of the subjects

Culture results	(N=71)
Positive, n (%)	
Gravis biovar	2 (2.8)
Mitis biovar	15 (21.1)
Negative, n(%)	54 (76.1)

diphtheria). Both groups were predominantly aged 5-9 years at the time of infection. Both groups also had relatively similar age characteristics. But the patients with confirmed diphtheria had a slightly narrower age range at the time of infection. Subjects' characteristics are summarized in **Table 2**.

Table 2. Characteristics of the subjects

Characteristics	Clinical diphtheria (n=71)	Confirmed diphtheria (N=17)
Sex, n (%)		
Male	29 (59.2)	7 (41.2)
Female	42 (40.8)	10 (58.8)
Age group, n (%)		
0-4 years	21 (29.6)	4 (23.5)
5-9 years	31 (43.7)	8 (47.1)
10-19 years	19 (26.8)	5 (29.4)
Age characteristics, n (%)		
Mean (SD), years	7.19 (4.289)	7.77 (4.2061)
Median, years	6	7
Minimum, years	1	3
Maximum, years	19	18

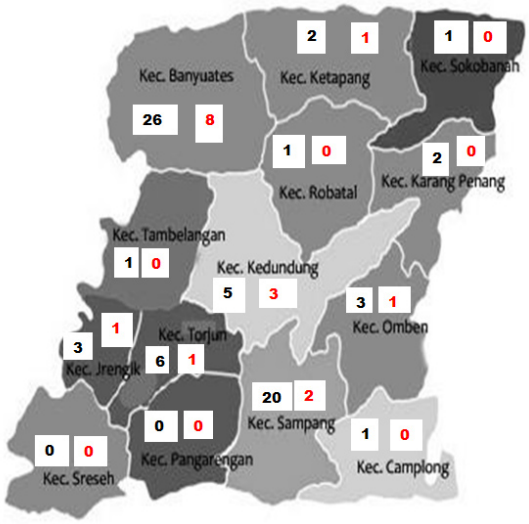

Figure 1. Map of diphtheria patient distribution by-district in the Sampang District. Left box (black font): number of patients with clinical diphtheria; Right box (red font): number of patients with confirmed diphtheria.

Diphtheria patients were found in 12 out of the total of 14 districts in the Sampang District. A large number of cases were found in Banyuates District and Sampang District. However, no patients were found in Sreseh and Pangarengan Districts, as shown in **Figure 1**.

There was a larger proportion of severe diptheria in patients without complete immunizations. However, Spearman's correlation test revealed no significant correlation between immunization status and the degree of diphtheria severity of in patients with clinical and confirmed diphtheria (**Table 3**).

Fisher's exact test revealed no significant correlation between immunization status and fatality status in diphtheria patients with clinical and confirmed diphtheria (**Table 4**).

Complications occurred in 26 out of 71 (36.6%) diphtheria patients. The majority of patients with complications had bullneck, as displayed in **Table 5**. From the total of 26 patients having complications of diphtheria, 8 of them belong to the group of 17 patients with confirmed diphtheria, which were analyzed further. Chi-square test revealed a significant correlation between immunization status and complications of patients with clinical diphtheria, but no correlation between immunization status and complications in patients with confirmed diphtheria (**Table 6**).

Table 3. Analysis of immunization status and degree of severity

Diphtheria status	Immunization status	Diphtheria severity			P value
		Mild	Moderate	Severe	
Clinical diphtheria	Complete, n(%) (n=16)	1 (6.3)	13 (81.3)	2 (12.5)	0.469[a]
	Incomplete, n(%) (n=16)	1 (6.3)	9 (56.3)	6 (37.5)	
	No immunization, n(%) (n=39)	2 (5.1)	26 (66.7)	11 (28.2)	
Confirmed diphtheria	Incomplete, n(%) (n=5)	1 (20.0)	3 (60.0)	1 (20.0)	0.610[b]
	No immunization, n(%) (n=12)	0 (0.0)	8 (66.7)	4 (33.3)	

[a]Spearman's correlation coefficient (rs) = 0.087; [b]rs = 0.133

Table 4. Analysis of immunization status and fatality

Diphtheria status	Immunization status	Fatality status		P value
		Survived	Died	
Clinical diphtheria	Complete, n(%) (n=16)	15 (93.8)	1 (6.2)	0.820
	Incomplete, n(%) (n=16)	14 (87.5)	2 (12.5)	
	No immunization, n(%) (n=39)	37 (94.9)	2 (5.1)	
Confirmed diphtheria	Incomplete, n(%) (n=5)	4 (80.0)	1 (20.0)	0.294
	No immunization, n(%) (n=12)	12 (100.0)	0	

Table 5. Types of complications in diphtheria patients

Complication, n	(n=26)
Bullneck only	23
Bullneck, shock, epistaxis	1
Shock, epistaxis	1
Shock, myocarditis	1
Total	26

low percentage of positive culture results (17/71, 23.9%) was also noted by Puspitasari *et al.* in Dr. Soetomo General Hospital, Surabaya, Indonesia, in which only 22.8% of patients had positive culture results.[14] In addition, Rusmil *et al.* found that only 1.9% of subjects had positive cultures during the diphtheria outbreak in Cianjur District, West Java Province, Indonesia.[15] The relatively low percentage of positive culture results in

Table 6. Analysis of immunization status and complications

Diphtheria status	Immunization status	Complications		P value
		No complications	With complications	
Clinical diphtheria	Complete, n(%) (n=16)	14 (87.5)	2 (12.5)	0.013
	Incomplete, n(%) (n=16)	6 (37.5)	10 (62.5)	
	No immunization, n(%) (n=39)	25 (64.1)	14 (35.9)	
Confirmed diphtheria	Incomplete, n(%) (n=5)	2 (40.0)	3 (60.0)	0.620
	No immunization, n(%) (n=12)	7 (58.3)	5 (41.7)	

Discussion

Of 71 subjects, 17 (23.9%) had positive culture results for *Corynebacterium diphtheriae*, with *mitis biovar* more common than *gravis biovar*. This finding was similar to those found in the studies of the 1990s outbreak in the newly independent states that formerly constituted the Soviet Union, in which mitis biovar predominated the culture results.[12,13] Two patients with gravis biovar were identified in our study. The first patient was identified in December 2011 and survived, but the second case was identified in July 2015 and died due to myocarditis. The diphtheria studies is due to the difficulty of growing Corynebacterium diphtheriae bacteria in the medium.[16] Limited laboratory facilities also contributed to the low percentage of subjects with positive culture results.[13] Since the Sampang District had inadequate facilities for culturing *Corynebacterium diphtheriae*, lab cultures were done at the Surabaya Laboratory of Health (*Balai Besar Laboratorium Kesehatan Surabaya*) in Surabaya, the capital of East Java Province.

In our study, both patient groups (clinical and confirmed diphtheria) had more female than male patients. Similarly, a Hyderabad, India study

had more female than male patients, ranging from pediatric to adult patients.[17] A multivariate analysis by Volkze et al. in Germany found that adult females had a weaker immune response in the form of lower antibody titers which also lasted for a shorter time than those in adult males. This weaker immune response made them 45% more susceptible to diphtheria than males.[18]

In our study, most patients were aged 5-9 years. This finding may be due to the waning of antibody titers from inadequate booster administration.[16] Diphtheria also occurred mostly in early childhood years (9 years and below) due to inadequate immune system performance.[16] School-aged children have increased frequency of contact with the disease-causing pathogen, leading to higher natural immunity to diphtheria.[19]

We found no correlation between immunization status and degree of diphtheria severity. A study during the outbreak in Buri Ram, Thailand also noted the absence of such a correlation.[20] Various studies showed that other factors, such as residential humidity, household density, and the wall type in houses had significant correlations with the severity of diphtheria.[21] Sampang District is endemic to diphtheria, so residents may have frequent contact with the disease-causing pathogen. Such a situation leads to higher natural immunity to diphtheria, enabling the diphtheria severity in patients to be compromised.[19] We did not measure patients' antibody levels, which might have shown a direct relationship between patient immunity and diphtheria severity.

We also found no correlation between immunization status and fatality of diphtheria patients. Previous studies have shown that low immunity due to poor nutrition, younger age, inadequate or absence of immunizations, and delayed treatment may increase the risk of death in diptheria patients.[22,23] However, immunization status was the sole variable, of those mentioned above (multifactorial), analyzed in this study. We also used the immunization status data from patient interviews, in addition to the Provincial Health Office data, which may have led to recall bias. Nevertheless, we consistently found that those with complete immunization status suffered less severe disease, and lower fatality, although the results were not statistically significant.

We found a statistically significant correlation between immunization status and complications in patients with clinical diphtheria, but not in those with confirmed diphtheria. The number of patients with confirmed diphtheria was significantly smaller than those with clinical diphtheria. Inadequate immunization may result in a higher risk of complications in diphtheria.[20] This is shown by a study of diphtheria among adult alcoholic patients in Sweden in which all patients with neurological complications had antibody titers below 0.01 IU/mL.[24] No similar study conducted on pediatric patients. Complete immunization was shown to increase the antibody titer in the recipients, possibly decreasing the probability of complications.[19] The absence of a correlation between immunization status and complications in patients with confirmed diphtheria was because none of the confirmed diphtheria cases had complete immunization status. Thus, in confirmed diphtheria cases, the risk of developing complications for incomplete and unimmunized patients was similarly higher compared to those with complete immunizations.

This study was limited as there were multiple sources of data: official medical records at the East Java Provincial Health Office, data obtained by the Diphtheria Research Team, and history-taking from diphtheria patients and their families. Data obtained from diphtheria patients and their families may have been subject to recall bias, causing inaccuracy of some parts of the data. In addition, we did not measure the antibody level against diphtheria toxin, so a more objective measurement could not be provided.

In conclusion, there is a significant correlation between immunization status and complications in patients with clinical diphtheria, but not in patients with confirmed diphtheria. This absence is likely because none of confirmed diphtheria cases have complete immunization for diphtheria.

Conflict of Interest

None declared.

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Original Article

Pattern and frequency of pediatric congenital heart disease at the Cardiac Research Institute of Kabul Medical University, Afghanistan

Abdul Muhib Sharifi

Abstract

Background Congenital heart disease (CHD) is the most common birth defect, with incidence of 0.7-0.9 live birth; it increases to 2-6% if first degree relative is affected. In Afghanistan majority of births take place at home and routine screening of neonates is not common, so true birth prevalence of CHD cannot be possibly calculated. Therefore, true prevalence of CHD in our population is unknown.

Objective To verify the current pattern and frequency distribution of congenital heart disease (CHD) at the Cardiac Research Institute of Kabul Medical University.

Methods This retrospective study was conducted in children aged 0-14 years, who underwent echocardiography for possible congenital heart disease from January 2015 to December 2016.

Results Of 560 patients who underwent echocardiography, 392(70%) had cardiac lesions. Congenital cardiac lesions were found in 235 (60% of those with lesions) patients, while 157 (40%) patients had rheumatic heart disease. Patients with CHD were further subdivided into acyanotic and cyanotic groups. The majority of acyanotic group had isolated atrial septal defect (55%) while the most common lesion in the cyanotic group was Tetralogy of Fallot (42%).

Conclusion Congenital heart defects are the most common heart disease in the pediatric population presenting at the Cardiac Research Institute of Kabul Medical University. Atrial septal defect (ASD) was the most common acyanotic defect, while Tetralogy of Fallot (ToF) is the most common cyanotic defect. **[Paediatr Indones. 2018;58:106-9; doi: http://dx.doi.org/10.14238/pi58.3.2018.106-9]**.

Keywords: *congenital heart disease; acyanotic; cyanotic CHD; echocardiography*

Congenital heart disease (CHD) is the most common birth defect, with an incidence of 7-9 per one thousand live births.[1] Pediatric CHDs are structural malformations of the cardiovascular system present at birth.[1] Congenital heart disease is a high contributor to the overall burden of pediatric cardiovascular diseases.[2] In developing countries like Afghanistan with very limited health facilities and poor access to the health centers, having a child with cardiovascular defects often results in social and economic problems that affect the entire family.[2] In the Western world, the incidence of CHD was reported to be 8/1,000 live births.[1,2] In Afghanistan, it has been difficult to obtain an accurate figure of the incidence of congenital heart disease. In Pakistan, a study reported a CHD prevalence of up to 6/1,000 live births.[3] In the neighboring country or India, another study reported a similar incidence.[4] In countries like Afghanistan, the true estimate of CHD prevalence is difficult to assess, as the majority of births occur at home and routine childhood

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screening is uncommon, due to financial, cultural, and religious issues. Data on immigrants from the Indian Subcontinent, however, showed a higher incidence of CHDs compared to the Caucasian population.[5]

Congenital heart defects could be isolated lesions or in combination with other cardiac malformations, and may also be associated with certain syndromes.[3] A systematic review mentioned, that in Asia, the highest total birth prevalence of CHD was 8/1,000 live births.[6] Other study has shown that in developing countries, the burden of CHDs continues to rise, due to increases in risk factors and etiological factors for such defects, including infection during pregnancy and older maternal age. The incidence of CHD in Afghanistan is likely much higher than what has been reported in the literature.[7] In developed countries, early diagnosis and suitable treatment has significantly increased the survival rate and has decreased mortality from 85% to 15% in CHD patients. As such, the number of adults with CHD has increased.[8]

In Afghanistan, few studies have been performed at the regional level to estimate the prevalence of CHD. Moreover, unfortunately, data on the prevalence at a general level needed to assess the burden of CHD nationwide is no available. In addition, there is no registry at the national level to approximate the total number of cases. Most past studies considered only the age of patients with CHD who visited the hospital, and the age at which the initial diagnosis was made.[9] Our study was performed at the Cardiac Research Institute of Kabul Medical University with the aim of determining the current frequency and pattern of congenital heart disease distribution.

Methods

A retrospective, cross-sectional study was done at the Cardiac Research Institute of Kabul Medical University from January 2015 to December 2016. Patients aged 0-14 years with echocardiography-confirmed structural heart disease were included in the study. Data were taken from medical records. The diagnosis of CHDs were based on trans-thoracic echocardiography, performed by two experienced echo cardiographer and the patients were reviewed by the consultant pediatric cardiologist, and their clinical presentation were recorded on their medical files.

Congenital heart defects are classified as cyanotic and acyanotic based on the presence and absence of cyanosis and direction of their shunts on echocardiography. We included ventricular septal defect (VSD), atrial septal defect (ASD), pulmonary stenosis (PS), patent ductus arteriosis (PDA), aortic stenosis (AS), and coarctation of the aorta (COA) as acyanotic, because clinically they had no cyanosis, and their shunts were left to right. Those who had right to left shunt we included as cyanotic CHD. In cyanotic group, clinically they had cyanosis which was recorded on their files, and they were included truncus arteriosis, tricuspid atresia, PDA (with right to left), transposition of the great artery (TGA), Tetralogy of Fallot (ToF), total anomalous pulmonary venous connection (TAPVC). Patients with insufficient medical records and those who had no confirmed echocardiography report were excluded. Data analysis was done with *Statistical Package for Social Sciences* (SPSS) software. This study was approved by the Ethics Committee of Pediatric Cardiology Department, Kabul Medical University, Afghanistan.

Results

Of 560 patients who underwent echocardiography, 392 (70%) had cardiac lesions, while 168 (30%) had normal findings. Congenital cardiac lesions were found in 235 (60% of those with lesions) patients, while 157 patients (40%) had rheumatic heart disease (RHD). Patients with CHD were further subdivided into cyanotic and acyanotic groups (**Table 1**).

The majority of the patients in the acyanotic group had isolated atrial septal defect (55%) (**Table 2**). Tetralogy of Fallot was the most common lesion in the cyanotic heart disease group (42.8%) (**Table 3**).

Table 1. The relative frequency and type of lesion in children with CHD

Type of cardiac lesion	N=392
Congenital heart disease, n(%)	235 (60)
Acyanotic CHD, n(%)	188 (80)
Cyanotic CHD, n(%)	47 (20)
Rheumatic heart disease, n(%)	157(40)

Table 2. Distribution of lesion types in patients with acyanotic heart disease (n=188)

Types of lesion, n(%)	Acyanotic CHD (n=188)	Compared to total CHD (n= 235)	Mean age (SD), years
VSD	53 (28.1)	53 (22.5)	2.3 (0.7)
ASD	103 (54.7)	103 (43.8)	4.7 (1.3)
PS	3 (1.5)	3 (1.27)	6.8 (1.2)
PDA	19 (10.1)	19 (8.08)	2.3 (0.7)
VSD+ASD	6 (3.1)	6 (2.5)	1.4 (0.6)
AS	2 (1.06)	2 (0.8)	7.1 (0.9)
COA	2 (1.06)	2 (0.8)	4.2 (1.8)

VSD=ventricular septal defect, ASD=atrial septal defect, PS=pulmonary stenosis, PDA=patent ductus arteriosis, AS=aortic stenosis, COA=coarctation of the aorta

Table 3. Distribution of lesion types in patients with cyanotic heart disease (n= 47)

Types of lesion, n(%)	Cyanotic CHD (n=47)	Compared to total CHD (n=235)	Mean age (SD), year
Truncus arteriosis	4 (8.5)	4 (1.7)	1.2 (0.8)
Tricuspid atresia	14 (29.8)	14 (5.9)	2.5 (0.4)
PDA (Eisenmenger syndrome)	2 (4.3)	2 (0.9)	6.5 (1.5)
TAPVC	4 (8.5)	4 (1.7)	1.3 (0.7)
TGA+VSD	3 (6.4)	3 (1.27)	1.7 (0.3)
ToF	20 (42.6)	20 (8.5)	2.8 (1.2)

TAPVC=total anomalous pulmonary venous connection, TGA=transposition of the great artery, ToF=Tetralogy of Fallot

Discussion

Congenital heart disease (CHD) is the most common birth defects in the pediatric population. This congenital defect is the leading cause of infant mortality and also a major risk factor in developing countries like Afghanistan, both in terms of morbidity and mortality.[1] Most of the children born with CHDs are expected to have normal, productive lives, when treated as early as possible. However, in developing countries, given the high incidence of birth asphyxia and various childhood infections, CHD remains neglected and often overlooked. In such settings, multiple factors including poverty, poor access to health care, lack of available trained staff, spiritual and social beliefs, lack of awareness about CHD and the absence of screening programs result in delayed diagnoses and poor outcome for children with CHD.[9] Early treatment of CHD may prevent a serious risk of avoidable mortality, morbidity, and handicaps.[10] The aim of this study was to verify the pattern and frequency of CHD in pediatric patients at the Cardiac Research Institute of Kabul Medical University.

In Afghanistan most births still take place at home, so it is nearly impossible to screen all the new borns for congenital anomalies. The data presented in this study were confined to hospital records, but there may be a large number of cases without access to a hospital or who died before reaching the hospital due to life threatening lesions. Hence, obtaining exact information on the prevalence and incidence of congenital heart disease in the pediatric population of Afghanistan remains a challenge. We found that among our CHD subjects, 20% had cyanotic heart disease and 80% had acyanotic heart disease, similar to results reported in both local and international studies.[2-12] The ASD and VSD were the most common lesions in our study subjects. Of our CHD subjects, we noted that 103/235 (43.8%) had ASD, which was similar to results from previous studies in neighboring Pakistan, 32.1% at the Punjab Institute of Cardiology (PIC), Lahore, 39.9% at the National Institute of Cardiology Karachi, and 38.16% at the Siddique Sadiq Memorial Trust Hospital, Gujranwala. The second most common congenital heart lesion found in our subject was isolated VSD, with prevalence of 22.5%. Similar prevalence were reported in the in the National Institute of Cardiovascular Disease (NICVD), Karachi, Karachi (18.2%), and Lahore (19.2%).[6] The 3rd most common pediatric cardiac lesion found in our study was PDA (8.08% of total CHD) which was seen 6.1 % of patients at NICVD, Karachi and 7.03% in Lahore. These figures clearly indicate that the pattern of congenital heart defects in three most common acyanotic CHD was similar in a neighboring country. Amongst cyanotic CHD patients, Tetralogy of Fallots was the highest at NICVD Karachi (24.3%) and PIC, Lahore (16.1%). Similarly, the most common cyanotic CHD in our study was also Tetralogy of Fallot.[10]

This study is important in two respects. Rheumatic heart disease was the most common cause of presentation to our hospital (40%). Recent literature involving community and school based studies from Pakistan has also confirmed that rheumatic heart

disease continues to be an important cause of morbidity and mortality in the pediatric population despite drastic drops in the incidence of rheumatic heart disease in the rest of the world.1[3-15]

Early detection and surgical intervention in developed countries have provided a significant chance of survival in children with CHD, but unfortunately in developing countries like Afghanistan the facilities for diagnosis and treatment of children with CHD are very limited and very expansive and beyond the reach of poor people. As such, many children die at early age, before diagnosis has been established. To improve the survival of children with CHD, it is necessary to diagnose and treat the condition as early as possible, by provision of diagnostic and surgical facilities at each province of the country.

Our study was performed over a period of two year in a teaching hospital, showing that CHD is still common in our society and constitutes a big health problem. The majority of CHD types in children up to 12 years of age are acyanotic followed by cyanotic heart disease. Ventricular septal defects and atrial septal defects were the major acyanotic CHDs, and Tetralogy of Fallot (ToF) was the major cyanotic CHD. Most of the information about morphology and hemodynamics can be obtained by 2D echo and Doppler examination of all infants and children suspected of having CHD, therefore, it is an essential tool for diagnosis, in addition to proper management to improve the survival of patients with various CHDs by medical or surgical intervention at the earliest possible age.

Conflict of Interest

Non declared.

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Original Article

Sleep disturbance scale for children as a diagnostic tool for sleep disorders in adolescents

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Abstract

Background Inadequate sleep may affect mental, emotional, and physical health, as well as the immune system. If sleeping time is not sufficient, then sleep disturbance may occur. Objective assessments of sleep quality can be done by polysomnography and actigraphy. Subjective assessments of sleep quality and quantity can be done with questionnaires or interviews. *The Sleep Disturbance Scale for Children* (SDSC) is a multi-dimensional sleep assessment questionnaire.

Objective To compare the SDSC to wrist actigraphy for assessing sleep quality in adolescents.

Methods We conducted a diagnostic study with a cross-sectional method, from March to April 2015 at elementary schools in Manado. The inclusion criteria were healthy adolescents aged 10 to 12 years, who agreed to fill the questionnaire and underwent wrist actigraphy. Data were analyzed using Chi-square test and 2 x 2 table to assess sensitivity, specificity, positive predictive value, and negative predictive value.

Results Of 60 adolescents, 31 were female and 29 were male, with a mean age of 11.39 years. The sensitivity of SDSC was 80.6%, specificity 37.9%, positive predictive value 58.1%, and negative predictive value 64.7%, when compared to wrist actigraphy as the gold standard of measuring sleep quality.

Conclusion The SDSC is a good screening tool for early detection of sleep disorders in adolescents. The Sleep Disturbance Scale for Children has a sensitivity of 80.6% and specificity of 37.9% for diagnosing sleep disturbances, as compared to the gold standard of wrist actigraphy. **[Paediatr Indones. 2018;58:9133-7; doi: http://dx.doi.org/10.14238/pi58.3.2018.133-7]**.

Keywords: *Sleep Disturbance Scale for Children; SDSC; adolescent; sensitivity; specificity*

While sleep is essential for every individual, sleep needs differ by age. The total amount of required sleep decreases gradually over childhood into adulthood.[1,2] The process of growth and development from intrauterine life, to childhood, to puberty, and to adulthood is continuous.[3] Sleep disturbances in adolescents may involve one or more basic mechanisms: inadequate sleep duration for age, disordered and fragmented sleep, or a discrepancy of sleeping time period. Sleep quality is often assessed by polysomnography and actigraphy. The wrist actigraph requires computer software and specialized training to interpret results.[5] For subjective assessment of quality and quantity of sleep, questionnaires or interviews can be used. *The Sleep Disturbance Scale for Children* (SDSC) is a sleep assessment questionnaire with advantages over other questionnaires because it includes multidimensional measurements.[4] The aim of this study was to compare the SDSC to wrist actigraphy for assessing sleep disorders in adolescents.

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Methods

We conducted a diagnostic study with a cross-sectional method from March to April 2015 in three elementary schools in Manado. Schools and subjects were selected by two-stage random sampling. Inclusion criteria were healthy adolescents aged 10 to 12 years, who were willing to answer the questionnaire and undergo wrist actigraphy. Subjects' parents provided written informed consent. Adolescents who were sick during wrist actigraphy, diagnosed and undergoing therapy for sleep disturbances, had chronic illnesses like hypertension, diabetes, or anemia, and with incomplete addresses or phone numbers were excluded from this study. The criterion for dropping out was an improperly attached wrist actigraph. The minimum required sample size was 57 subjects. This study was approved by the Ethics Committee for Research, Sam Ratulangi University Medical School.

The Sleep Disturbance Scale for Children used in this study assessing six categories of sleep disorders (1) sleep breathing disorders, (2) start and maintain sleep disorders, (3) consciousness disorders, (4) sleep-awake transition disorders, (5)) excessive somnolent disorders, and (6) hyperhidrosis during sleep. Direct interviews were conducted by researchers on parents and children who were included in the study. The score of sleep disturbance defined based on intensity or frequency of each category above: score 1 for never, score 2 for seldom (≤1-2 times/month), score 3 for sometimes (1-2 times/week), 4 for frequent (3-5 times/week), and 5 for always (daily). The cut off point of total score was 39, hence sleep disturbance was diagnosed if the total score was more than 39.[6]

Subjects were diagnosed with sleep disturbances if one or more of three sleep parameters showed abnormal patterns in wrist actigraphy. The three parameters are effectivity of sleeping less than 85%, sleep onset latency (SOL) of more than 20 minutes, and wake after sleep onset (WASO) of more than 40 minutes. Data were analyzed using Chi-square test and 2 x 2 table to show sensitivity, specificity, positive predictive value, and negative predictive value of the SDSC compared to wrist actigraphy as the gold standard.

Results

A total of 60 adolescents were included after randomization, with the mean age of 11.39 (SD 0.64) years (**Table 1**). Subjects' mean sleep time began at 21:19 hours, mean time to get up was 5:28 hours, and mean total sleep time was 7 hours 31 minutes. In our study, 19 subjects with sleep disturbances watched TV for more than 3 hours per day. Forty-three subjects were diagnosed to have sleep disturbance using the SDSC (**Table 2**). Using wrist actigraphy, 31 subjects were found to have sleep disturbances, including 10 subjects with sleep efficiency of less than 85% and WASO more than 40 minutes. The mean sleep onset latency was 14.4 (SD 21.3) minutes; mean sleep efficiency was 85.2 (SD 6.9) %, and mean wake after sleep onset was 41.3 (18.9) minutes (**Table 3**). The diagnostic test to compare the SDSC to wrist actigraphy showed sensitivity of 80.6%, specificity

Table 1. Characteristics of subjects

Variables	N=60
Mean age (SD), years	11.39 (0.64)
Sex, n(%)	
Female	31 (51.7)
Male	29 (46.3)
Mean body weight (SD), kg	33.7 (8.89)
Mean body height (SD), cm	140.25 (7.6)

Table 2. Subjects' sleep assessment by the SDSC

SDSC results	N=60
No sleep disturbance, n (%)	17 (28.3)
Sleep disturbance, n (%)	43 (71.7)
Type of sleep disturbance, n (%)	
Disorders of initiating and maintaining sleep	15 (34.9)
Sleep breathing disorders	7 (16.3)
Disorders of arousal	4 (9.3)
Sleep-wake transition disorders	9 (20.9)
Disorders of excessive somnolence	7 (16.3)
Sleep hyperhydrosis	1 (2.3)

Table 3. Quality of sleep measured by wrist actigraphy

Variables	N=60	95%CI
Mean sleep onset latency (SD), min	14.4 (21.3)	0.0 to 103.7
Mean sleep efficiency (SD), %	85.2 (6.9)	65.1 to 93.4
Mean wake after sleep onset (SD), min	41.3 (18.9)	10 to 127.2

of 37.9%, positive predictive value of 58.1%, and negative predictive value of 64.7% (**Table 4**). There was no significant difference in results from SDSC compared to wrist actigraphy in determining the presence of sleep disturbance (P=0.055).

and mean total sleep time was 7 hours 31 minutes. A previous study in Hong Kong found that adolescents aged 12-19 years had a mean total sleep time of 6.3 hours, beginning at 00:03 hours and waking at 06:33 hours.[12] Another study in South Korea found that

Table 4. Comparison between wrist actigraphy and SDSC to assess sleep disturbance

		Wrist actigraphy		Total
		Sleep disturbance	No sleep disturbance	
SDSC	Sleep disturbance	25	18	43
	No sleep disturbance	6	11	17
	Total	29	60	

Discussion

Sleep is a regular resting condition with reduced characteristic gestures and decreased level of awareness of surroundings. It is reversible and rapid. Sleep is essential, but the need for sleep differs according to age.[7-9] Adolescence is a phase of dynamic development, characterized by accelerated physical, mental, emotional, and social development.1 Early adolescence starts from the age of 10-12 years, with dramatically increased physical and psychological growth. Sleep quality is closely linked to the overall welfare of adolescents, including their physical, emotional, cognitive, and social health.[1]

To subjectively assess quality and quantity of sleep, questionnaires or interviews are often used in epidemiological studies. The questionnaire is easy touse and analyze. The SDSC is a sleep assessment questionnaire with advantages over other questionnaires, as it is a multi-dimensional assessment.[10-13]

Wrist actigraphy is the standard benchmark to evaluate sleep disturbances in adolescents. Adolescent body movement is recorded by the actigraph tool and used to determine the presence or absence of sleep disturbances. A sleep disorder is diagnosed when wrist actigraphy records at least one of three parameters: sleep efficiency < 85%, sleep onset latency > 20 minutes, and WASO > 40 minutes.[11]

In our study, subjects' mean sleep time began at 21:19 hours, mean time to get up was 5:28 hours, young people aged 10-14 years had a mean sleep time of 6.6 hours per day.[13]

Wrist actigraphy revealed that 31 children experienced sleep disturbances, including 16 (51.6%) girls. Wrist actigraphy began at 18:00 hours and continued until the morning of the next day. Data were collected and processed by Actiware software.

Using the SDSC, we found that 43 (71.7%) adolescents experienced sleep disturbances. The most common type of sleep disorder was initiating and maintaining sleep (insomnia) in 15 (34.9%) adolescents. Haryono *et al.* in Jakarta found that 62.9% of adolescents aged 12-15 years experienced sleep disturbances using the SDSC. The most common sleep disorders were sleep-wake transition disorders, suggesting that sleep disturbances are commonly found in adolescents.[14]

Disorders of initiating and maintaining sleep (insomnia) were seen in 34.9% of subjects with sleep disturbances by SDSC. This result was greater than that of Hysing *et al.* in Norway, who reported the prevalence of insomnia in adolescents aged 16-18 years to be 23.8%.[15] In a 2012 study on 384 adolescents aged 13-18 years, Dohnt et al. found that the prevalence of insomnia was 34.6%.[16] The most common causes of insomnia in adolescents is the excessive use of technology, such as a mobile phone and television.[17]

We compared subjects' SDSC results to their wrist actigraphy results and found that the SDSC sensitivity was 80.6% and specificity was 37.9%.

Chi-square analysis revealed no significant difference between the two tests (P=0.055). Natalita *et al.* obtained SDSC sensitivity and specificity of 71.4% and 54.5%, respectively. They also reported that McNemar test revealed no significant difference in SDSC and wrist actigraphy (P=0.832).[10] However, the specificity in our study was 37.9%, suggesting that clinical judgment is still required from the examiner to determine the absence of a sleep disorder. Hence, the SDSC questionnaire can be used to screen for sleep disturbances in adolescents, as an easy-to-use and low cost tool.

The SDSC is easier to use than the gold standard. Wrist actigraphy is expensive and requires specialized training and software to interpret the results. *The Sleep Disturbance Scale in Children* is a multi-dimensional assessment questionnaire with advantages over other questionnaires because it includes a multi-dimensional measurement.

Limitations of this study were that the subjects' activity levels were not monitored, nor was more than one night of sleep (wrist actigraphy) monitored. Thus, we are unable to speculate on the causes of sleep disturbances in our subjects.

In conclusion, the *Sleep Disturbance Scale for Children* has a sensitivity of 80.6% and specificity of 37.9% for diagnosing sleep disturbances, as compared to the gold standard of wrist actigraphy. Therefore, the SDSC questionnaire is a good screening tool for early detection of sleep disturbances in adolescents.

Conflict of Interest

None declared.

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Original Article

Ultraviolet-related ocular problems in children living on the coast of Southwest Sumba, Indonesia

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Abstract

Background A previous study has shown a high proportion of visual impairment and blindness in the adults of Southwest Sumba, Indonesia due to ultraviolet-radiation (UVR)-related ocular problems, such as cataract and pterygium. Currently, there is no data regarding the effect of ultraviolet (UV) exposure on children and its future implications.

Objective To seek the predisposing factors of UVR ocular problems in the children of Southwest Sumba.

Methods A population-based cross-sectional study was conducted in Perokonda Village, Southwest Sumba in May 2017. A total of 337 children <16 years old were examined for ocular problems by ophthalmologists. Subjects with ocular problems were then treated accordingly.

Results Visual acuity was normal in 98.2% of subjects. Visual impairment and blindness were found in 1.2% and 0.3% of subjects, respectively. Ocular problems were found in 38%, consisting of conjunctival pigment deposits (60.2%), pinguecula (15.6%), and pterygium (13.3%). The UVR ocular problems constituted 33.8% of all ocular problems, comprising conjunctival pigment deposits (22.8%), pinguecula (6%), and pterygium (5%).

Conclusion The proportion of UVR ocular problems in the children of Perokonda village is 34.5%, the most common of which being conjunctival pigment deposits. Such deposits may be early signs of UVR ocular problems. This study serves as a platform to highlight the possible relationship between pigment deposits and future UVR ocular problems, which warrants further study. **[Paediatr Indones. 2018;58:128-32; doi: http://dx.doi.org/10.14238/pi58.3.2018.128-32]**.

Keywords: *ocular problems; ultraviolet; pigment deposit; children*

Blindness and visual impairment are major health concerns around the globe. Approximately 285 million people are visually impaired, with 39 million suffering from blindness, most of whom live in low-income settings.[1] *The World Health Organization* (WHO) has established *VISION 2020*: Right to Sight as an initiative to eliminate blindness and visual impairment, especially in underdeveloped areas, by bringing eye care to remote areas and ensuring the availability of eye care to all.[2]

Indonesia is a developing country located along equatorial lines with constant exposure to the sun, hence harboring an increased risk of ocular problems caused by excessive ultraviolet (UV) exposure.[3] Uneven distribution of health care facilities and personnel combined with unnoticed ocular problems amplify the magnitude of this problem.

Perokonda Village in Southwest Sumba District is a remote coastal village with excessive sun exposure where most community members work as fishermen with low socioeconomic status and low educational

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levels. Moreover, the people of Perokonda village spend the majority of their time outside, partly owing to the lack of facilities for indoor activities. Accumulation of UV exposure in early stages of life may contribute to the increased risk of developing eye diseases such as pterygium, cataract, and pinguecula. A 2016 survey to determine the distribution of ocular problems in adults in Southwest Sumba found that cataract and pterygium were prevalent (12.8% and 10.7%, respectively), possibly owing to the constant exposure to UV without protection.[4]

Although the prevalence of blindness and visual impairment in UV-exposed individuals has been reported in adults, it has not been studied in children. This study aimed to determine the predisposing factors to UV-related (UVR) ocular problems in children with constant UV exposure.

Methods

This population-based, cross-sectional study was conducted in Perokonda Village in May 2017. The children were examined by a team of four ophthalmologists and two general practitioners from the Universitas Indonesia Medical School, Jakarta. Prior notices regarding the event were given to local residents through the chiefs of the village and banners. Examination was conducted in the local elementary school, which was converted into a clinic. The ophthalmologists diagnosed eye problems by history taking and ophthalmologic examinations. Prior to the examination, parents were given information regarding the study and asked for consent. All children aged less than 16 years in Perokonda Village who came and followed the examination process until completion were included in the study. Each subject and the parents were informed consent prior to the examination.

Visual acuity was measured using a *Plusoptix® S12C* mobile autorefractor (Plusoptix Inc., Atlanta, Georgia). In accordance with WHO standards, we classified visual acuity as blindness (<3/60), severe visual impairment (<6/60), moderate visual impairment (<6/18), or mild/absent visual impairment (≥6/18). Children who could not read or show directions were examined using a fix and follow test. An anterior segment examination was performed using *KOWA SL-15* portable slit lamp (Kowa Company Ltd., Nagoya), and a posterior segment examination using *Welch Allyin* standard ophthalmoscope (Welch Allyn, Skaneateles Falls, New York). Data were analyzed using *SPPS version 20*. We used a 95% confidence interval (CI) and a significance level of $P<0.05$.

After diagnoses were made, subjects were treated accordingly. Refraction errors were corrected using corrective lenses, given one month following the examination. Eye infections were treated with antibiotics. One subject with blindness due to corneal staphyloma was referred to Dr. Cipto Mangunkusumo Hospital, Jakarta for evisceration surgery. The study protocol has been approved by the Medical Research Ethics Committee of the Universitas Indonesia Medical School.

Results

A total of 337 subjects between six months and 16 years of age were included in the study. There were 166 (49.3%) males and 171 (50.7%) females. Subjects' educational level ranged from preschool to senior high school; most were in primary school (62%). Most of the parents were fishermen (67.7%) and farmers

Table 1. Demographic characteristics of study subjects

Characteristics	n (%)
Gender	
Male	166 (49.3)
Female	171 (50.7)
Level of education	
No education	40 (11.8)
Preschool	9 (2.7)
Kindergartern	33 (9.8)
Primary school	209 (62.0)
Junior high school	37 (11.0)
Senior high school	9 (2.7)
Parent's occupation	
Fisherman	228 (67.7)
Farmer	53 (15.7)
Teacher	26 (7.7)
Employee	16 (4.7)
Others	14 (4.2)
Parent with prescription glasses	
None	300 (89.0)
Father	14 (4.1)
Mother	15 (4.5)
Both	8 (2.4)

(15.7%). The demographic characteristics of our subjects are shown in **Table 1**.

Table 2 shows the subjects' visual acuity. There were 331 children (98.2%) with normal visual acuity in both eyes. Six children had impaired visual acuity. Moderate visual impairment was caused by refractive error and papillary atrophy due to traumatic optic neuropathy. All cases of severe visual impairment were caused by refractive errors. One child had unilateral blindness due to corneal staphyloma (**Table 3**).

Ocular problems were found in 128 children (38%). The most common problems were conjunctival pigment deposits (60.2%), pinguecula (15.6%), and pterygium (13.3%), followed by others such as conjunctivitis, microcornea, scleral pigment deposits, and limbal pigment deposits (**Table 3**). Two subjects were diagnosed with pinguecula and conjunctival pigment deposits, nine with pinguecula and pterygium, and two with pterygium and conjunctival pigment deposits.

Table 4 shows that the prevalence of UVR ocular problems in Perokonda village was 34.5%. The most common UVR ocular disease was conjunctival pigment deposit (22.8%), followed by pinguecula (6%) and pterygium (5%). Most of the pterygiums, pinguecula, and pigmented deposits were located in the interpalpebral fissure.

Table 2. Visual acuity of children in Perokonda Village

Classification	OD, n(%)	OS, n(%)	ODS, n(%)	Overall, n(%)
Normal vision	0	0	331 (98.2)	331 (98.2)
Moderate visual impairment	2 (0.6)	1 (0.3)	0	3 (0.9)
Severe visual impairment	0	1 (0.3)	1 (0.3)	2 (0.6)
bLINDNESS	0	1 (0.3)	0	1 (0.3)

OD=right eye; OS=left eye; ODS=both eyes

Table 3. Proportion of ocular problems in the children of Perokonda Village

Diagnosis	OD, n(%)	OS, n(%)	ODS, n(%)	Subjects, n(%)
Moderate visual impairment	2 (1.6)	1 (0.8)	-	3 (2.4)
Traumatic optic neuropathy	1 (0.8)	-	-	1 (0.8)
Myopia + astigmatism*	1 (0.8)	1 (0.8)	-	2 (1.6)
Severe visual impairment	-	1 (0.8)	1 (0.8)	2 (1.6)
Congenital retinal dystrophy	-	-	1 (0.8)	1 (0.8)
Hyperopia$^{\Delta}$	-	1 (0.8)	-	1 (0.8)
Blindness				
Corneal staphyloma	-	1 (0.8)	-	1 (0.8)
Conjunctivitis	1 (0.8)	1 (0.8)	3 (2.4)	5 (3.9)
Conjunctival pigment deposit	11 (8.6)	10 (7.8)	56 (43.8)	77 (60.2)
Limbal pigmentation	0	0	1 (0.8)	1 (0.8)
Scleral pigmentation	1 (0.8)	0	0	1 (0.8)
Pinguecula	5 (3.9)	3 (2.3)	12 (9.4)	20 (15.6)
Pterygium	1 (0.8)	2 (1.6)	14 (10.9)	17 (13.3)
Microcornea	0	0	1 (0.8)	1 (0.8)
Total	21 (16.4)	19 (18.4)	88 (68.8)	128 (100)

*two subjects had anisometropia, $^{\Delta}$subject had esotropia alternans in both eyes

Table 4. Proportion of UVR ocular problems in children of Perokonda Village, 2017

Diagnosis	OD, n(%)	OS, n(%)	ODS, n(%)	Subjects, n(%)
Conjunctival pigment deposit	11 (3.3)	10 (3)	56 (16.5)	77 (22.8)
Limbal pigmentation	0	0	1 (0.3)	1. (0.3)
Scleral pigmentation	1 (0.3)	0	0	1 (0.3)
Pinguecula	5 (1.5)	3 (0.9)	12 (3.6)	20 (6)
Pterygium	1 (0.3)	2 (0.6)	14 (4.2)	17 (5.1)
Total	18 (5.4)	15 (4.5)	83 (24.6)	116 (34.5)

Discussion

The province of East Nusa Tenggara, where Perokonda Village is situated, has the second highest prevalence of blindness in Indonesia. The magnitude of the problem is enhanced due to its challenging geographical terrain, lack of health- and eye care services, poverty, and low education. Children in Perokonda Village spend most of their time doing outdoor activities. The high prevalence of normal visual acuity in these children (98.2%) may be attributable to the lack of near-vision activity, such as watching television and playing computer games. However, unprotected outdoor activities also carry the risk of UVR ocular diseases.

UV exposure has been associated with the occurrence of eyelid malignancies (such as basal cell carcinoma and squamous cell carcinoma), photokeratitis, climatic droplet keratopathy, pterygium, and cortical cataract.[5] However, evidence of the association between UV exposure and development of pinguecula, nuclear and posterior subscapular cataract, ocular surface squamous neoplasia, and ocular melanoma is not fully established.

On the molecular level, UV creates active free radicals, which attacks various macromolecules.[6] The abundance of free radicals will lead to oxidative DNA damage. Double-stranded DNA (dsDNA) damage is affected by different expressions of dsDNA break repair genes such as XRCC2, XRCC3, RAD50, and RAD51.[7] Moreover, one of the most common genetic markers of human neoplastic growth p53 is highly expressed the UVR population. The p53 gene, which functions as a cell cycle regulator and has a role in DNA repair and synthesis, cell differentiation, and apoptosis, is mutated in UVR population.[7]

The high prevalence of pterygium and pinguecula in Perokonda Village suggests the significant involvement of UV radiation in the development of those diseases. Pterygium is a type of benign uncontrolled growth of the conjunctiva that lays over the sclera, which impairs the visual acuity and triggers an inflammatory process. Pterygium mostly affects people with higher exposure to sunlight and other factors such as sand and wind.[8] Pinguecula is a UV radiation exposure-related fibro-fatty degenerative change in the bulbar conjunctiva within the palpebral aperture. A study in Sumatra, Indonesia reported a high prevalence of pterygium due to the abundance of sun exposure during outdoor activities.[9] Therefore, UV, sand, and wind may contribute to the progression of pterygium and pinguecula in Perokonda village.

A previous study reported that the prevalence of pterygium in Perokonda Village adults is 10.7%.[4] In contrast, we found a prevalence of 5% in children from the same village. These findings suggest that there may be an increasing trend in the proportion of UVR eye diseases from time to time. Furthermore, accelerating degenerative process due to persistent UVR radiation could lead to early cataract formation. In Perokonda village adults, the prevalence of cataract is 12.8%, dominated by those aged >40 years (12.1%). Among those with cataract, fisherman was the most common occupation (6.4%), while only 0.3% were students.[4] Based on those results, we suggested that the high prevalence of pterygium and cataract in adults might be initiated by high UV radiation from the early stages of life.

Yellowish brown pigment deposits in the conjunctiva (60.2%) are common in children of Perokonda Village. Such pigment deposits are different from pterygium, pinguecula, nevus, or other melanoma characteristics. There are no feeding veins or signs of inflammation around the deposits, which are usually found in the pterygium and pinguecula. The yellowish brown pigment tends to appear like several droplets in certain areas of the ocular surface. Moreover, the yellowish brown pigmentation, pinguecula, and pterygium are located in the nasal and temporal area of the eyes, which might show that the areas with high UV exposure tend to develop pigments more easily than areas protected by the eyelids. A study on pterygium and UV-treated conjunctival cells using immunochemistry and the Northern blot procedure found a significantly higher expression of elastin compared to normal cells, due to elastodysplasia and elastodystrophy processes.[10] Elastin is a major component of connective tissue in large arteries, lung, skin, and ligaments, and provides elasticity in those tissues.[10] Due to the high proportion of yellowish pigment deposits in the population of Perokonda Village, we hypothesize that these deposits are one of the most apparent early sign of UVR eye diseases.

Prevention of UVR ocular diseases are achieved by simple behavioral changes, such as wearing appropriate clothing, hats, and UV-blocking spectacles or sunglasses.[3] The most effective method to limit

UVR problems is by avoidance of exposure. Wearing clothing made of thick synthetic materials, hats with wide brims, contact lenses, and sunglasses are effective methods of avoidance.[3] To obtain in-depth knowledge about the yellowish pigment, microscopic samples are needed.

These findings highlight the effect of UV exposure to ocular health from the early stages of life, and stress the importance of UV protection and public awareness in ocular health. In children of Perobatang Village, UV exposure has led to conjunctival pigment deposit, pinguecula, and pterygium, which may contribute to the risk of visual impairment and blindness. Further study to explain this phenomenon is required.

The main limitation of this study was the design of the study was a cross-sectional study hence there is no correlation that can be elucidated. To draw a more conclusive relationship between sun exposure and UVR eye problems in such areas, a prospective cohort study is needed. Despite the limitations, this study is the first to explore the possible outcome of UV exposure from early stages of life.

In conclusion, 38% of children in Perokonda Village suffer from ocular problems, consisting of conjunctival pigment deposit (60.2%), pinguecula (15.6%), and pterygium (13.3%). The proportion of UVR ocular problems in the children of Perokonda village is 34.5%, mostly comprised of conjunctival pigment deposit (22.8%), pingecuela (6%) and pterygium (5%). The yellowish pigment deposit is thought to be an early sign of UVR ocular problems.

Conflict of Interest

None declared.

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Original Article

Menstrual cycle patterns of Indonesian adolescents

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Abstract

Background Adolescents often experience menstrual irregularity in the first few years after menarche. Abnormal menstrual cycles may increase the risk of polycystic ovary syndrome.

Objective To determine the menstrual cycle patterns of adolescent females in Indonesia and associated factors.

Methods This cross-sectional study was conducted at two senior high schools in Surakarta, Central Java, from September to October 2016. Subjects were healthy female students whose parents have given written informed consent. Girls taking hormonal drugs or with chronic diseases were excluded. Subjects filled questionnaires on menstrual cycle, diet, ethnicity, and physical activity. All subjects underwent anthropometric measurements (height and weight). Kruskal-Wallis test was used for data analysis.

Results Four hundred and forty-four subjects met the inclusion criteria. Mean age at menarche was 12.27 (SD 1.08) years. Mean menstrual cycle length was 31.1 (SD 6.5) days. Abnormal menstrual cycle occurred in 30.6% of subjects (24.5% oligomenorrhea, 5.9% polymenorrhea, and 0.2% amenorrhea). Ethnicity (Javanese, Chinese, or Arab) was significantly associated with menstrual cycle category ($P<0.05$). Girls with Chinese ethnicity having the largest proportion of oligomenorrhea. Other factors (body mass index, age, age at menarche, nutritional status, physical activity, and fat intake) were not found to be associated with menstrual cycle abnormalities.

Conclusion Menstrual abnormalities, especially oligomenorrhea, are common in Indonesian adolescent girls. Oligomenorrhea is more frequent in girls of Chinese ethnicity, compared to those of Javanese or Arab ethnicity. **[Paediatr Indones. 2018;58:101-5; doi: http://dx.doi.org/10.14238/pi58.3.2018.101-5]**.

Keywords: *menstrual cycle; adolescent; oligomenorrhea; polymenorrhea*

The length of a normal menstrual cycle ranges from 21 to 35 days and may vary within as well as between individuals. Factors such as nutrition, ethnicity, age at menarche, physical activity, body mass index, and hormones are considered to contribute to pubertal development, but their potential effects on the length of the menstrual cycle remain unclear. Menstrual cycle irregularities, be it oligomenorrhea, polymenorrhea, or amenorrhea, may arise due to pregnancy, infection, malignancy, trauma, hormonal disturbance, emotional stress, vigorous physical activity, or dietary problems.[1,2]

In the first few years after menarche, adolescents commonly have irregular menstrual cycles. Such irregularity is physiological in most girls, but may be associated with an increased risk of polycystic ovary syndrome and ovarian dysfunction in others. In some conditions, adolescents, parents, and clinicians need to be educated on what constitutes a normal menstrual cycle. Adolescent girls should take note of their cycles so that data is on hand when the need arises for a clinician to follow up an abnormality of their menstrual cycle, for example in the preventive monitoring for polycystic ovary syndrome.[3,4] We aimed to determine the menstrual

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cycle patterns of female students in Surakarta, Central Java, Indonesia, and determine potential risk factors for menstrual cycle abnormalities.

Methods

Girls from two high schools in Surakarta (SMA Negeri 3 and SMA Islam Diponegoro) were screened from September 2016 to October 2016. Study subjects were healthy female students whose parents have given written informed consent and had experienced at least three menstrual cycles before the study period. We excluded girls who took hormonal drugs or had chronic diseases, such as asthma, kidney disease, diabetes mellitus, thyroid abnormalities, or cancer. Subjects were asked to complete a questionnaire regarding menstrual cycle and ethnicity data. Oligomenorrhea was defined as a menstrual cycle longer than 35 days; polymenorrhea was defined as a cycle of less than 21 days; and amenorrhea was defined as having no menstrual period for at least three months in a row.

Weight and height were measured using a digital standardized scale and a standardized microtoise, respectively. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of body height in meters. Each measurement was performed three times and a mean of the three measurements was calculated for each subject. The subjects' nutritional status were categorised based on body mass index for age (BMI-for-age) according to the *WHO Child Growth Standards*.[5] Normal weight was defined as BMI-for-age between -1 standard deviations of the mean (-1SD) and +1SD. Overweight, obesity, and underweight were defined as BMI-for-age >+1SD but ≤+2SD, >+2SD, and <-1SD, respectively.

Subjects were asked to complete questionnaires on reproductive health, physical activity (*International Physical Activity Questionnaire*/IPAQ),[6] and dietary habits (*Semi-Quantitative Food Frequency Questionnaire*/SQ-FFQ).[7] Physical activity level was categorized as vigorous (≥1500 METs min.week), moderate (600-1500 METs min.week) and low (<600 METs min. week) (METs min.week = metabolic equivalent task minutes per week). Fat intake was obtained from the SQ-FFQ and categorized as less than once a week, once to twice a week, three times a week, or ,more than three times a week. Data were processed using *SPSS 20.0* and analyzed using the Kruskal-Wallis test. This study was approved by the Health Research Ethics Committee of Dr. Moewardi General Hospital/Sebelas Maret University Medical School, Surakarta.

Results

Out of 463 girls who were screened, 444 were included as study subjects. Subject characteristics are described in **Table 1**. The majority of subjects were Javanese (341 subjects; 76.8%). The subjects' BMI ranged from 14.71 to 32.64 kg/m2. Sixty-seven subjects (15.1%) were overweight or obese. Subjects' mean age at menarche was 12.27 (SD 1.08) years.

Mean menstrual cycle length was 31.1 (SD 6.5) days, ranging from 12.0 to 96.5 days. Abnormal menstrual cycles were found in 136 (31.6%) subjects, consisting of oligomenorrhea in 24.5%, polymenorrhea in 5.9%, and amenorrhea in 0.2% (**Table 2**). The

Table 1. Characteristics of subjects

Characteristics	(N=444)
Mean age (SD), years	16.5 (0.96)
Ethnicity, n (%)	
Javanese	341 (76.8)
Chinese	39 (8.8)
Arab	64 (14.4)
Nutritional status, n (%)	
Underweight	114 (25.7)
Normal	263 (59.2)
Overweight/obese	67 (15.1)
Mean BMI (SD), kg/m^2	21.54 (4.66)
Physical activity level, n (%)	
Low	143 (32.2)
Moderate	105 (23.6)
Vigorous	196 (44.2)
Mean age at menarche (SD), years	12.27 (1.08)

Table 2. Menstrual cycle category and mean menstrual cycle length

	(N=444)
Menstrual cycle category, n (%)	
Normal	308 (68.4)
Polymenorrhea	26 (5.9)
Oligomenorrhea	109 (24.5)
Amenorrhea	1 (0.2)
Mean menstrual cycle length, days (SD)	31.1 (6.5)
Shortest	12.0
Longest	96.5

associations between menstrual cycle category and possible risk factors (nutritional status, ethnicity, physical activity, fat intake, BMI, age, and age at menarche) are shown in **Table 3**. The proportions of menstrual cycle categories differed significantly between ethnic groups. Significant differences were observed between Javanese and ethnic Chinese girls, as well as between Javanese and ethnic Arab girls ($P<0.05$; Mann-Whitney test). No significant difference was found between ethnic Chinese and Arab girls ($P=0.068$; Mann-Whitney test). The proportion of oligomenorrhea in ethnic Chinese, Arab, and Javanese subjects was 53.8%, 37.5%, and 18.8%, respectively. Current age, age at menarche, BMI, physical activity, nutritional status, and intake of foods high in fats were not significantly associated with menstrual cycle category ($P>0.05$).

College of Obstetricians and Gynecologists Committee on Adolescent Health Care (ACOG) reported in 2006 that in American adolescents, the average menstrual cycle length was 32.2 days and the menstrual cycle interval ranged from 21 to 45 days.[1] Studies in America, Turkey, Ghana, and Japan reported varying menstrual cycle lengths ranging from 20 to 45 days.[2,11-13] Girls with menstrual cycles of more than 90 days should be evaluated for underlying conditions.[4]

There were 136 (31.6%) subjects with abnormal menstrual cycles, the majority having oligomenorrhea. Smaller incidence rates of menstrual cycle abnormalities have been reported in the United States (2-5%)2 and Italy (9%).[9] In Turkey the reported incidence of oligomenorrhea was 5.3%, in contrast to 24.5% in our study.[14] On the other hand, studies in Malaysia, Turkey, Hyderabad, Central India, and Ghana have reported prevalences

Table 3. Association between menstrual cycle category and risk factors

	Normal	Polymenorrhea	Oligomenorrhea	Amenorrhea	P value
Nutritional status, n (%)					0.589*
Underweight	77 (67.6)	7 (6.1)	30 (26.3)	0 (0)	
Normal	188 (71.4)	12 (4.6)	62 (23.6)	1 (0.4)	
Overweight	43 (64.2)	7 (10.4)	17 (25.4)	0 (0)	
Physical activity, n (%)					0.892*
Low	98 (68.5)	10 (7.0)	35 (24.5)	0 (0)	
Moderate	75 (71.4)	5 (4.8)	25 (23.8)	0 (0)	
Vigorous	135 (68.9)	11 (5.6)	49 (25.0)	1 (0.5)	
High-fat food intake, n (%)					0.089*
<1x/week	29 (60.4)	2 (4.2)	17 (35.4)	0	
1-2x/week	141 (74.6)	9 (4.8)	39 (20.6)	0	
3x/week	19 (76)	2 (8)	4 (16)	0	
>3x/week	119 (65.4)	13 (7.1)	49 (26.9)	1 (0.6)	
Ethnicity, n (%)					<0.001*
Javanese	253 (74.2)	23 (6.7)	64 (18.8)	1 (0.3)	
Chinese	16 (41.0)	2 (5.2)	21 (53.8)	0 (0)	
Arab	39 (60.9)	1 (1.6	24 (37.5	0 (0	
Mean BMI, kg/m2 (SD)	21.5 (4.6)	22.1 (4.9)	21.6 (5.0)		0.894*
Mean age, years (SD)	16.5 (1.0)	16.5 (1.0)	16.7 (0.9)		0.579*
Mean age at menarche, years (SD)	12.2 (1.1)	12.3 (1.0)	12.4 (1.1)		0.397*

*Kruskal Wallis test

Discussion

The mean age at menarche of our subjects was similar to that found in other studies.[8-11] Subjects' mean menstrual cycle length was 31.1 (SD 6.5) days. Individuals with the shortest and longest menstrual cycles were of Javanese ethnicity. *The American Academy of Pediatrics* (AAP) and the *American* of menstrual cycle irregularities comparable to our study.[11,12,14-17] Although menstrual irregularity in the first 5 years after menarche is considered to be physiological, the risk of polycystic ovary syndrome (PCOS) and ovarian dysfunction may evolve in the first years after menarche. A recent report stated that menstrual cycle irregularity in adolescents was not correlated to oligoanovulation, but was associated with ovarian volume.[3,18,19]

Oligomenorrhea is one of the clinical features of PCOS.[3] In our study, oligomenorrhea was the most prevalent menstrual cycle abnormality, with the proportion being the lowest in the Javanese ethnic group, significantly different from ethnic Chinese and Arab subjects. Studies on ethnicity-specific rates of PCOS in Indonesian adolescents have been limited, if any. It remains to be determined whether the higher incidence of oligomenorrhea in ethnic Chinese girls translates into a higher incidence of PCOS. Further observation should be done in girls with oligomenorrhea, since a menstrual cycle abnormality persisting more than two is a risk factor for PCOS.[4]

Many studies have investigated the role of ethnicity in early or post-menopausal women, but studies in adolescents have been limited. The proportion of menstrual cycle abnormalities in Indonesian ethnic Chinese girls in our study was higher than the proportion of irregular menstrual cycles in Malaysian ethnic Chinese girls (59% *vs.* 38.4%, respectively).[17] However, it was unclear whether the definition of "irregular menstrual cycle" in the Malaysian study was comparable to our definition of menstrual cycle abnormalities. In the Pokhara Valley, Nepal, ethnicity was also found to have a role in adolescent menstrual patterns. The proportion of irregular menstrual cycles in this population was 64.2%, with the ethnicity group classified as "Muslim/ others" having the highest proportion (73.9%), followed by the Hill Ethnic group (71.7%), Hill caste (59.8%), and Dalit (36.4%). The reason for this difference was not explained.[20]

We found no associations between menstrual cycle category and nutritional status, physical activity, fat intake, BMI, age, or age at menarche. In contrast, a Malaysian study reported that age and being within two years of reaching menarche, in addition to smoking and suicidal behavior, were significantly associated with irregular menstrual cycles. The same study did not find a significant association between ethnic group, dieting behavior, BMI, alcohol use, and physical exercise.[17] In agreement with our results, a study in Japanese young adults found that food habits and lifestyle were not associated with menstrual cycle length.[13] In contrast to our results, a study in adolescent girls in Hyderabad, India found that BMI was significantly associated with menstrual patterns.[15] A Serbian study also noted correlations between menstrual irregularity and height, weight, BMI, and obesity.[18] Zhang *et al.* reported that older adult women tend to have a shorter menstrual cycle length, and that among women with a shorter cycle length (<27 days) there was a higher frequency of abnormal BMI (underweight or overweight).[21] Among women in menopausal transition, obesity was found to be associated with longer menstrual cycles.[22]

A limitation of our study was that our subjects were only from a single urban area; the menstrual pattern of rural adolescents might have different characteristics. Our convenience sampling method may have led to disproportionate ethnic representation among the subjects. In addition, there may have been recall bias for some of the data, including menstrual cycle length, age at menarche, physical activity, and diet data, as we used only questionnaires to collect these data. However, we used the *Lie-Score Minnesota Multiphasic Personality Inventory* (L-MMPI) questionnaire to reduce bias caused by dishonesty.[23] Moreover, we did not assess ethnic differences in the rates of PCOS in our subjects, which would have taken our study a step further in clinical relevance.

In conclusion, there is a high prevalence of menstrual cycle abnormalities in Indonesian adolescents (31.6%). Clinicians may want to consider our data in determining what constitutes a normal menstrual cycle in Indonesian adolescents. Any abnormalities should be followed up monitor the risk of polycystic ovary syndrome. Current age, age at menarche, BMI, physical activity, and fat in the diet are not associated with menstrual cycle abnormality. Significant ethnic differences exist in the rate of menstrual cycle abnormalities.

Conflict of interest

None declared.

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Original Article

Sitting height, sitting height/height ratio, arm span, and arm span-height differences of healthy adolescents

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Abstract

Background Sitting height, sitting height/height ratio (SHR), arm span, and arm span-height difference (AHD) are indices to diagnose conditions of disproportion. Reference data on sitting height, SHR, arm span, and AHD for Indonesian children are limited.

Objective To compile reference data on sitting height, SHR, arm span, and AHD in Indonesian adolescents, and to compare these indices for boys and girls at various ages.

Methods A population-based survey was conducted from August 2016 to November 2017 in three high schools in Surakarta, Central Java, Indonesia. A convenience sampling method was employed to recruit healthy adolescents without history of chronic disease, history of physical trauma, and/or physical disabilities. All subjects underwent anthropometric measurements (height, weight, sitting height, and arm span), and their ethnic origins were noted. The lambda-mu-sigma (LMS) method was used for reference construction.

Results Of 639 subjects, 42% were male. Body mass index (BMI) values were similar between males and females. Mean height, weight, sitting height, and arm span of males were greater than those of females. The mean male and female SHRs were 51.1 (SD 1.6) % and 51.0 (SD 1.6) %, respectively (P=0.36), while the mean AHDs were 4.2 (SD 4.5) cm and 3.4 (SD 4.1) cm, respectively (P=0.02). The formula to estimate height based on arm span in males was [height = (0.78 x arm span) + 32.14] in cm. The formula in females was [height = (0.66 x arm span) + 50.59] in cm.

Conclusion There was no significant difference in SHR between male and female adolescents. However, males have significantly larger mean AHD than females. We provide references on sitting height, SHR, arm span, and AHD in male and female adolescents. **[Paediatr Indones. 2018;58:138-45; doi: http://dx.doi.org/10.14238/pi58.3.2018.138-45]**.

Keywords: *sitting height; sitting height/height ratio; arm span; arm span-height difference; adolescent*

Normal growth is essential for children. The routine anthropometric indices used to evaluate growth are height, weight, and head circumference. Subsequent advanced measurements are done when an abnormal result, e.g., short or tall stature, is found. These advanced measurements include upper-lower segment ratio, arm span, and other investigations into possibilities of organic diseases or dysmorphology.[1]

To assess for growth disorders, reference data on body proportion, such as sitting height, sitting height/height ratio (SHR), and arm span-height difference (AHD) are needed. The SHR varies among children of different ethnicities, ages, and sex. Body disproportion may be due to genetic disease or syndrome, such as short stature homeobox (SHOX) defects, Turner syndrome, idiopathic short stature, and achondroplasia.[2] Body proportion has also been correlated with some environmental factors, such as nutrition, lifestyle, and chronic disease. In addition, past studies have found a correlation between SHR and body mass index (BMI), a marker of obesity.[3,4] There

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is also evidence that adults with disproportionate body measurements are at risk for cardiovascular disease and impaired glucose metabolism.[5] Arm span measurement is useful for some syndromes, such as Marfan syndrome and achondroplasia. Both short and long extremities may lead to an abnormal AHD. A positive correlation between arm span and stature is helpful for predicting height in disabled individuals.[1,2]

Reference data for SHR, arm span, and AHD are needed for diagnosing growth disorders.[1] To date, we have limited national reference data for Indonesian children. Hence, we aimed to compile reference data on SHR and AHD among healthy adolescents in Surakarta, Indonesia.

Methods

This survey was conducted in three high schools (2 senior high schools and 1 junior high school) from August 2016 to November 2017 in Surakarta, Central Java, Indonesia. We asked for students who presented at their schools during study periods, to enroll this study (soliciting subjects). We obtained parental written informed consent for all participants. Inclusion criteria were male and female students aged 11 to 19 years. We excluded children with a history of chronic disease, physical trauma (from interview), or physical disability (from physical examination).

Subject characteristics were collected from school data records, including birth date, sex, and parents' ethnicities. If the data were incomplete, subjects were interviewed. If both parents were of the same ethnicity, subjects were categorized as being of Javanese, Chinese, or Arab ethnicity; otherwise, they were categorized as mixed/others.

All subjects underwent anthropometric measurements (height, weight, sitting height, and arm span) in triplicate by trained personnel. Each subject was measured three times by the same person and the mean of the three measurements was calculated. Height and sitting height were measured using a wall stadiometer (*Stature Meter 2M GEA*) to the nearest 0.1 cm with the subject facing the examiner. Subjects were asked to stand without shoes for height measurement and to sit for sitting height measurement. Body weight was measured using a digital scale (*Seca Clara 803*, Germany) to the nearest 0.1 kg. The SHR was calculated by dividing sitting height by height and expressed as percentage. The AHD was calculated by subtracting height from arm span in centimeters.

We reported means, standard deviations (SD) and percentages for descriptive data. Males and females were reported separately. We extrapolated the formula for height based on arm span by linear regression analysis. Reference charts were constructed using the the lambda-mu-sigma (LMS) method by *Cole6* with the help of *LMS Chartmaker Light version 2.54* software (Harlow Healthcare, Tyne & Wear, United Kingdom) for fitting and smoothing. Lambda, mu, and sigma represent the skewness (power transformation), median, and coefficient of variation from the Box-Cox transformation. We hypothesized that SHR and AHD were different between male and female. This study was approved by the Health Research Ethics Committee of Dr. Moewardi General Hospital/Sebelas Maret University Medical School, Surakarta.

Results

Six hundred and thirty-nine subjects were enrolled in this study. Subject characteristics are summarized in **Table 1**. The comparison of mean SHR and mean AHD between male and female subjects can be seen in **Table 2**.

The formulae for predicting male and female height based on arm span are described in **Table 3**. There is a strong correlation between height and arm span in both sexes (P=0.00). Charts depicting height, sitting height, SHR, body mass index, arm span, and AHD in males and females are shown in **Figure 1**. SHR patterns are similar in males and females, peaking in mid-adolescence, then decreasing. Male and female AHD patterns are also similar to each other, with the lowest values seen in mid-adolescence. The LMS parameters and SDs (-2SD and +2SD) for height, sitting height, SHR, and arm span in males and females are shown in **Tables 4** and **Table 5**. In males, some age groups had negative skewness values for SHR of <-2, while in females, the skewness in many age groups were <-2 or >2. **Table 6** describes the results from ethnic Chinese' subjects.

Table 1. Baseline characteristics of subjects

Characteristics	Total (N=639)	Male (n=266)	Female (n=373)
Mean age (SD), years	15.7 (1.6)	15.3 (1.7)	16.0 (1.5)
Mean height (SD), cm	157.7 (8.5)	162.8 (9.0)	154.1 (5.8)
Mean weight (SD), kg	53.4 (13.5)	56.7 (15.0)	51.1 (11.7)
Mean BMI (SD), kg/m^2	21.4 (4.6)	21.3 (4.9)	21.5 (4.5)
Mean sitting height (SD), cm	80.4 (4.5)	83.0 (4.5)	78.5 (3.5)
Mean arm span (SD), cm	161.4 (9.9)	166.9 (10.3)	157.5 (7.4)
Ethnicity, n (%)			
Javanese	481 (75.3)	213 (80.1)	268 (71.8)
Chinese	48 (7.5)	21 (7.9)	27 (7.2)
Arab	60 (9.4)	1 (0.4)	59 (15.8)
Others	50 (7.8)	31 (11.7)	19 (5.1)

Table 2. Sitting height/height ratio and arm span-height difference comparisons between male and female subjects

	Male (n=266)	Female (n=373)	P value
Mean SHR (SD), %	51.1 (1.6)	51.0 (1.6)	0.36
Mean AHD (SD), cm	4.2 (4.5)	3.4 (4.1)	0.02

greater than mean female corresponding values, although the mean age of the male group was lower. However, mean BMI and SHR were not significantly different between males and females. The mean heights of both male and female subjects in our study were within -2SD to +2SD of those found in an earlier study in Indonesian children,[7] but were below the WHO growth reference means.[8] We found mean SHRs of 51.1% in males and 51.0% in females. This ratio was slightly lower than that found in Dutch, Turkish, Chinese, and Spanish adolescents, which ranged from 52% to 53%.[1,9-11] Our ethnic Chinese subjects had mean SHRs similar to the overall mean

Table 3. Formulae to predict height based on arm span in males and females

	Formula	r	R^2	P value
Male	Height = (0.78 x Arm span) +32.14	0.90	0.81	0.00
Female	Height = (0.66x Arm span) +50.59	0.84	0.70	0.00

Table 4. LMS parameters and SDs for height, sitting height, SHR, and arm span in males

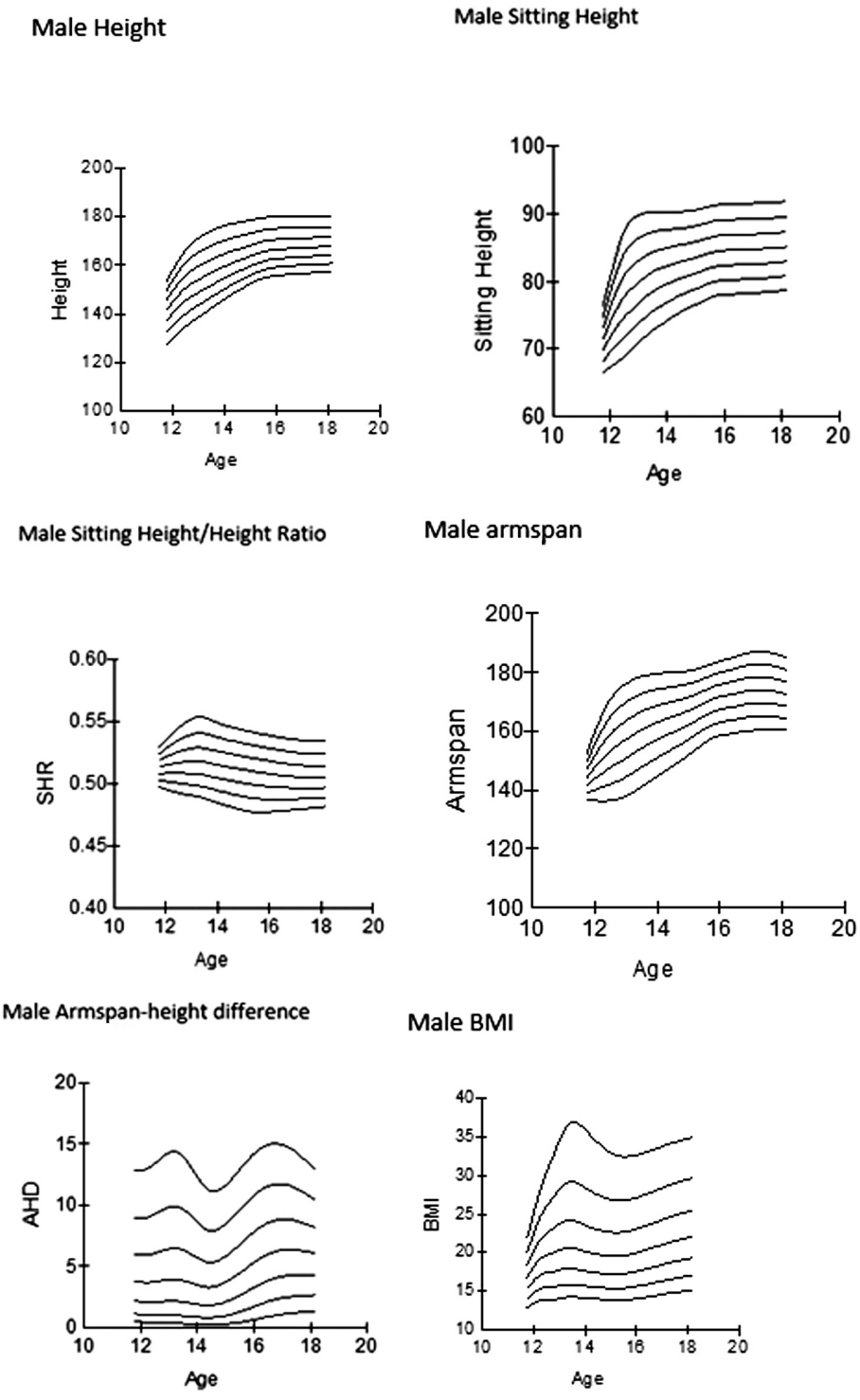
Age	Height					Sitting Height					Sitting Height/Height Ratio					Armspan				
(years)	L	S	M	-25D	25D	L	S	M	-25D	25D	L	S	M	-25D	25D	L	S	M	-25D	25D
12	2,52	0,05	144,78	129,83	157,70	1,15	0,04	73,92	67,27	80,48	-0 52	0,02	0,51	0,50	0,54	0,35	0,04	147,56	136,36	159,35
12,5	1,15	0,05	150,59	134,68	166,25	0,24	0,06	77,76	68,88	87,49	-198	0,03	0,52	0,49	0,54	0,75	0,06	153,30	136,44	170,64
13	0,04	0,05	154,06	138,60	171,16	0,14	0,06	79,83	70,83	89,80	-2 76	0,03	0,52	0,49	0,55	1,13	0,06	157,15	137,95	176,05
13,5	-0,71	0,05	156,88	142,31	174,19	0,75	0,05	81,43	72,78	90,30	-2 26	0,03	0,52	0,49	0,55	1,58	0,06	160,46	141,05	178,59
14	-1,38	0,05	159,43	145,97	176,26	1,11	0,05	82,28	74,19	90,28	-0 71	0,03	0,52	0,48	0,55	1,93	0,05	162,93	144,56	179,55
14,5	-1,95	0,04	161,63	149,35	177,46	1,10	0,04	82,96	75,52	90,33	0,76	0,03	0,51	0,48	0,55	2,14	0,05	164,91	148,19	179,89
15	-2,31	0,04	163,67	152,30	178,45	0,90	0,04	83,52	76,55	90,56	1,56	0,03	0,51	0,48	0,54	2,28	0,04	166,95	151,92	180,42
15,5	-2,36	0,04	165,51	154,60	179,53	0,62	0,04	84,22	77,54	91,09	1,56	0,03	0,51	0,48	0,54	2,29	0,04	169,57	155,93	181,92
16	-2,18	0,04	166,45	155,76	179,90	0,46	0,04	84,59	78,06	91,41	0,89	0,03	0,51	0,48	0,54	2,14	0,04	171,65	158,44	183,79
16,5	-2,03	0,04	166,85	156,24	179,99	0,44	0,04	84,63	78,12	91,44	-010	0,03	0,51	0,48	0,54	1,96	0,04	172,78	159,37	185,26
17	-182	0,03	167,26	156,73	180,06	0,40	0,04	84,74	78,26	91,53	-112	0,03	0,51	0,48	0,54	1,65	0,04	173,80	160,20	186,73
17,5	-174	0,03	167,38	156,88	180,06	0,36	0,04	84,84	78,40	91,62	-2 09	0,03	0,51	0,48	0,53	1,50	0,04	173,85	160,37	186,83
18	-148	0,03	167,68	157,28	179,96	0,28	0,04	85,03	78,65	91,78	-2 97	0,03	0,50	0,48	0,53	1,04	0,04	172,94	160,30	185,55

L= lambda= skewness (power transformation); S= sigma= coefficient of variation; M= mu=median

Discussion

We present anthropometric data of adolescents in Surakarta, Central Java, Indonesia. Mean male body height, weight, sitting height, and arm span were

A


Male Height
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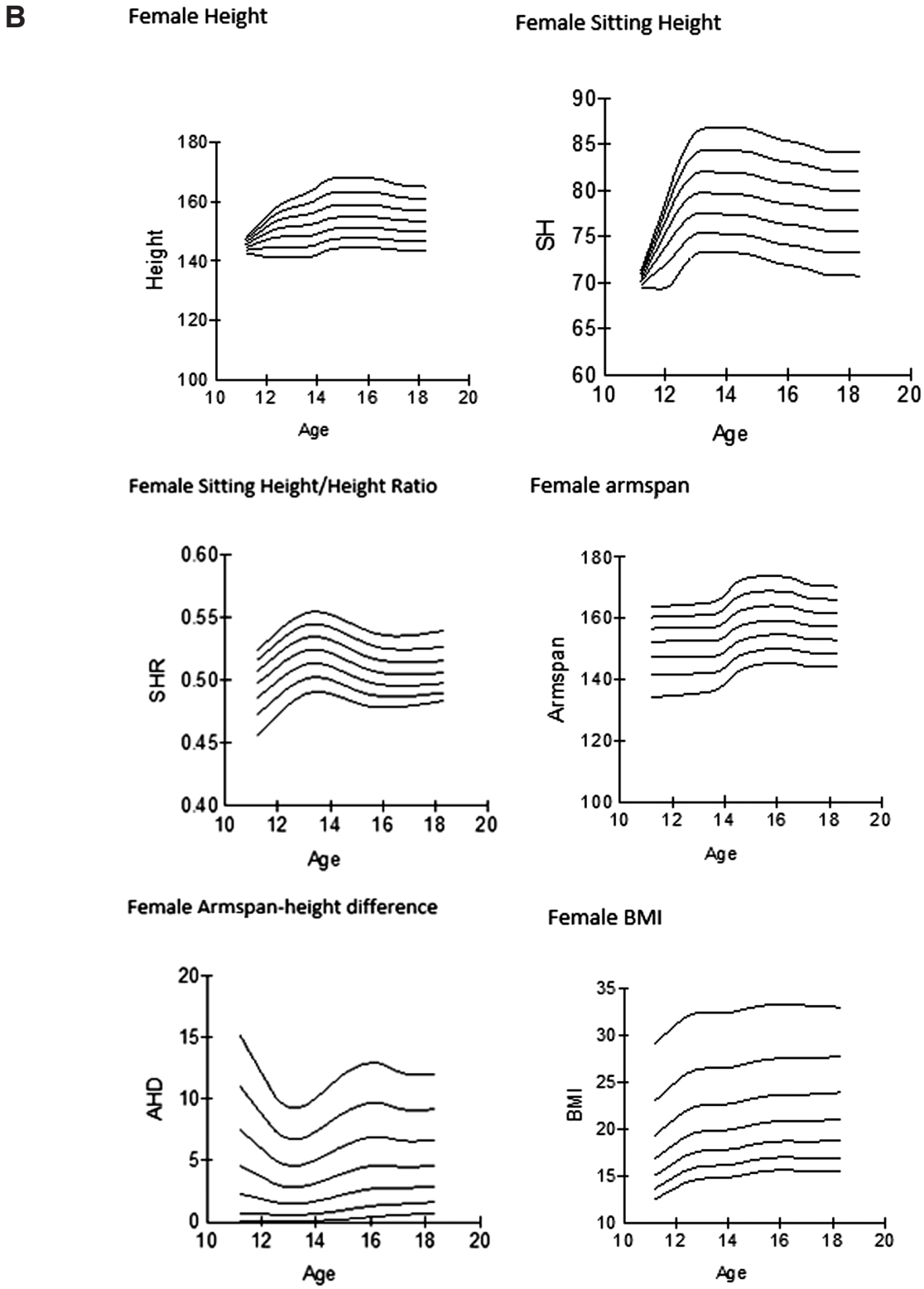

Figure 1. Charts for height, sitting height, SHR, arm span, AHD, and BMI in males (A) and females (B)

Table 5. LMS parameters and SDs for height, sitting height, SHR, and arm span in females

Age	Height					Sitting Height					Sitting Height/Height Ratio					Armspan				
(years)	L	S	M	-25D	25D	L	S	M	-25D	25D	L	S	M	-25D	25D	L	S	M	-25D	25D
11,5	11,83	0,01	147,24	142,23	150,88	17,06	0,02	72,38	69,20	74,22	6,80	0,03	0,50	0,46	0,53	5,75	0,04	152,46	134,46	163,87
12	8,57	0,02	149,56	141,56	155,23	10,44	0,03	75,53	69,09	79,03	5,71	0,03	0,51	0,47	0,54	5,38	0,05	152,47	134,79	164,10
12,5	5,59	0,03	151,07	141,14	158,68	4,43	0,04	78,24	71,35	83,52	4,60	0,03	0,52	0,48	0,55	4,95	0,05	152,49	135,15	164,37
13	3,36	0,03	151,69	141,02	160,84	0,78	0,04	79,66	73,12	86,31	3,50	0,03	0,52	0,49	0,55	4,42	0,05	152,49	135,57	164,70
13,5	1,53	0,03	151,96	141,12	162,40	-0,21	0,04	79,71	73,31	86,81	2,58	0,03	0,52	0,49	0,55	3,63	0,05	152,51	136,22	165,20
14	-0,56	0104	152,83	142,12	164,85	-0,38	0,04	79,62	73,24	86,80	1,85	0,03	0,52	0,49	0,55	2,29	0,05	153,98	138,62	167,59
14,5	-198	0,04	154,33	143,83	167,51	-0,42	0,04	79,56	73,18	86,76	1,21	0,03	0,52	0,49	0,55	0,94	0,05	157,19	142,61	171,86
15	-2,20	0,04	154,77	144,38	168,00	-0,33	0,04	79,24	72,84	86,41	0,58	0,03	0,51	0,48	0,54	0,46	0,05	158,52	144,36	173,40
15,5	-2,13	0,04	154,77	144,44	167,83	0,23	0,04	78,78	72,26	85,75	-0,03	0,03	0,51	0,48	0,54	0,56	0,04	159,19	145,19	173,76
16	-2,10	0,04	154,73	144,43	167,72	0,75	0,04	78,55	71,90	85,34	-0,70	0,03	0,51	0,48	0,54	0,69	0,04	159,33	145,42	173,62
16,5	-2,00	0,04	154,57	144,33	167,36	1,21	0,04	78,37	71,60	85,02	-1,59	0,03	0,51	0,48	0,54	0,76	0,04	159,19	145,36	173,31
17	-165	0,04	153,94	143,86	166,14	1,97	0,04	78,04	71,07	84,45	-2,74	0,03	0,50	0,48	0,54	0,97	0,04	158,06	144,62	171,54
17,5	-137	0,04	153,58	143,60	165,38	2,35	0,04	77,86	70,79	84,16	-4,10	0,03	0,51	0,48	0,54	1,08	0,04	157,54	144,31	170,69
18	-125	0,03	153,43	143,50	165,06	2,40	0,04	77,84	70,75	84,13	-5,54	0,03	0,51	0,48	0,54	1,10	0,04	157,45	144,26	170,53

L=lambda=skewness (power transformation); S=sigma=coefficient of variation; M=mu=median

Table 6. Anthropometric measurements on subjects of Chinese ethnicity

	Total (n=48)	Male (n=21)	Female (n=27)	P value
Mean age (SD), years	16.0 (1.4)	15.8 (1.6)	16.2 (1.1)	0.35
Mean height (SD), cm	159.1 (9.1)	164.0 (9.0)	155.3 (7.2)	0.00
Mean weight (SD), kg	55.4 (13.2)	58.4 (15.1)	53.0 (11.2)	0.16
Mean body mass index (SD), kg/m2	21.8 (4.5)	21.6 (4.9)	21.9 (4.2)	0.81
Mean sitting height (SD), cm	80.8 (4.0)	83.0 (3.9)	79.1 (3.2)	0.00
Mean sitting height/height ratio (SD), %	50.8 (1.1)	50.6 (0.9)	51.0 (1.2)	0.24
Mean arm span (SD), cm	162.8 (11.4)	168.4 (11.9)	158.5 (8.9)	0.00
Mean arm height difference (SD), cm	3.7 (4.6)	4.4 (5.0)	3.2 (4.3)	0.39

SHRs (**Table 6**), but lower than the mean SHR of Chinese adolescents in China.1[2] The mean SHR in late adolescence in our study was 50% to 51%. Our population had lower SHRs than that in a study by Galloway, who reported that the mean SHR in early adults (18 years old) was 53.4% in males and 53.8% in females.[3]

Other studies have noted that the SHR nadir occurred at an earlier age in females than in males.[1,10] This observation may be due to the earlier onset of puberty in females.[1,10] We did not assess the lowest SHR in our study, since we did not include subjects less than 11 years. Our findings were similar to reports from Turkish, Japanese, and Dutch studies that SHR was slightly increased in mid-pubertal age.[1,10,13] We observed peak median SHR at mid-pubertal age (age of 12.5-14.0 years old) for both males and females (52%); after this period SHR declined to 51% by the end of puberty. We can assume that at mid-pubertal age, growth in trunk length exceeds that of limb length; the opposite happens in late puberty. A similar pattern was observed in a Japanese study,[13] but not in Dutch and Turkish studies.[1,10] On the other hand, the SHR of Mozambique adolescents was relatively constant from early to late puberty, i.e., there was no peak SHR.[14] We also found that SHRs were similar between male and female adolescents. However, this was not the case in Turkish and Chinese studies.1[0,12]

Linear regression analysis revealed a strong correlation between arm span and stature in both males and females. A previous study by Wongsodjaja *et al.* on children in the nearby city of Semarang, Indonesia, also found strong correlations, with correlation coefficients (r) of 0.956 in boys and 0.972 in girls ($P < 0.001$ in both sexes).[15] Strong correlations between height and arm span were also found in adult males

and females in Nigeria, Montenegro, and India.[16-18] In the Indonesian elderly, Fatmah obtained an r of 0.765 in elderly men and 0.609 on elderly women ($P<0.05$ in male and female).[19] These results indicate that height and arm span strongly correlate at almost all ages, from children to the elderly. Wongsodjaja et al. obtained the following formulae in 12-year-olds: [height=31.881 + (0.773 x arm span)] for males and [height=50.476 + (0.648 x arm span)] for females.[15] These formulae are similar to those we have obtained. In the elderly in Jakarta, Indonesia, Fatmah obtained the following equations: [height=60.16 + (0.603 x arm span)] for men and [height=75.23 + (0.47 x arm span)] for women.[19]

In our study, subjects had longer mean arm span than height. However, this comparison differed between countries. Similar results were reported by Brown *et al.* in adults in New York,[20] Zverev *et al.* in Malawi,[21] Rai *et al.* in Ellisras rural children in Rajasthan,[22] and Goon *et al.* in Nigerian young adults.[16] In contrast, South African and Turkish children had longer height than arm span.[22,23]

In our study, mean arm span and AHD in females were lower than in males. The mean AHDs in our study were longer than those reported in another Indonesian study (less than 2 cm in both sexes) and in Turkish adolescents (less than 3 cm in both sexes).[15,24]

A limitation of our study was that subjects may not have been representative of all Indonesian adolescents because of the convenience sampling method, although we had a variety of ethnic origins (Javanese, Chinese, and Arab). We need more subjects from different geographical locations and ethnicities to obtain nationwide Indonesian references for sitting height, SHR, and AHD. Data from children of different socio-economic backgrounds are also needed, as all our subjects were from an urban area. Furthermore, our data was limited to mid-pubertal adolescent ages, so we were unable to assess for changes in SHR during early puberty. We did not perform Tanner staging; this might have biased our results. In addition, arm span measurements from prepubertal age are needed to determine the AHD from childhood.

In conclusion, we have obtained references for sitting height, SHR, and arm span in adolescents. The LMS parameters and SD values for sitting height, SHR, and arm span found in our study can be used to evaluate body disproportions in Surakarta adolescents. In our study population, there is no significant difference in SHR between male and female adolescents, but the mean AHD of males is longer than that of females. Further studies with a larger sample size involving a more diverse geographical and ethnic population, as well as inclusion of prepubertal and early pubertal children is needed, in order to provide more inclusive references for SHR and AHD in prepubertal and early pubertal children.

Conflict of Interest

None declared.

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Case Report

West syndrome and mosaic trisomy 13: a case report

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Trisomy 13, or Patau syndrome, is a rare chromosomal disorder characterized by a triad of cleft lip and palate, postaxial polydactyly, and microphthalmia, with an incidence ranging between 1/5,000 and 1/20,000 births.[1] Most patients (80%) with Patau syndrome have complete trisomy 13. Mosaic trisomy 13 is very rare; it occurs in only 5% of all patients with the trisomy 13 phenotype.[2] Trisomy 13 is a clinically severe entity, and 90 to 95% of patients born with this syndrome do not survive beyond one year of life. However, patients with mosaic trisomy 13 usually have longer survival and less severe phenotype compared to patients with complete trisomy 13. Malformations mainly affect midline development, with a high frequency of central nervous system involvement. The presence of central nervous system malformations is important as a predictive factor of survival.[1,3] It is well known that the incidence of epilepsy is higher in children with Patau syndrome than in the general population, and West syndrome or infantile spasms have been rarely reported in these children.[1,4,5] Prior to our report, there has been no case report of West syndrome associated with mosaic trisomy 13. The association of West syndrome with trisomy 13 is considered a symptomatic West syndrome because of preexisting psychomotor development delay and the poor prognosis in most of these children.[6] We report here the first case of West syndrome in a girl with mosaic trisomy 13 and discuss the clinical characteristics and prognosis of this association.**[Paediatr Indones. 2018;58:146-50; doi: http://dx.doi.org/10.14238/pi58.3.2018.146-50]**.

Keywords: *West syndrome; Patau syndrome; mosaic trisomy 13; prognosis*

The Case

A 4-month-old girl followed from birth for mosaic trisomy 13 developed repetitive flexor spasms, and was hospitalized in the Department of Paediatrics. She was the seventh child of healthy, non-consanguineous parents with 3 girls and 3 boys in good health. Her mother was 39 year old, the course of pregnancy was normal, and antenatal ultrasounds showed no anomalies. She was delivered vaginally at 42 weeks of gestation without incident. Apgar scores at 1 and 5 min were 8 and 9, respectively. She had dysmorphic features suggesting Patau syndrome with microcephaly, hypertelorism, upslanting palpebral fissures, epicanthal folds, broad nasal bridge, bilateral cleft lip and palate, and short neck (**Figure 1**). Transfontanellar ultrasound, abdominal ultrasound, and echocardiogram were normal. The cytogenetic examination of lymphocytes demonstrated a mosaicism of 47, XX, + 13 [6] /46, XX [10].

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Her psychomotor development at 3 months was abnormal with hypotonia and poor head control. Thyroid function was normal. At 4 months, she had flexor spasms several times a day, occurring in series, psychomotor developmental delay, and axial hypotonia. The EEG showed hypsarrhythmia. She was diagnosed as having West syndrome, and treated with Vigabatrin at a dose of 150 mg/kg/day. Vigabatrin therapy was immediately effective with good clinical control of spasms, but the EEG monitoring after one month was unchanged showing persistent hypsarrhythmia. Brain magnetic resonance imaging (MRI) showed lobar holoprosencephaly and callosal agenesis (**Figure 2**).

Because of her severe psychomotor delay, she received nasogastric tube feedings at home and the cleft lip and palate were untreatable. The first intervention of the cleft lip and palate was made at 16 months of age, but the post-operative course was marked by dropping of sutures. After a follow-up of 2 years, the patient had hypotonia, feeding difficulties, severe growth retardation, microcephaly, severe developmental delay, and tetraparesia. Her spasms disappeared with Vigabatrin. She died of pneumonia at the age of 26 months.

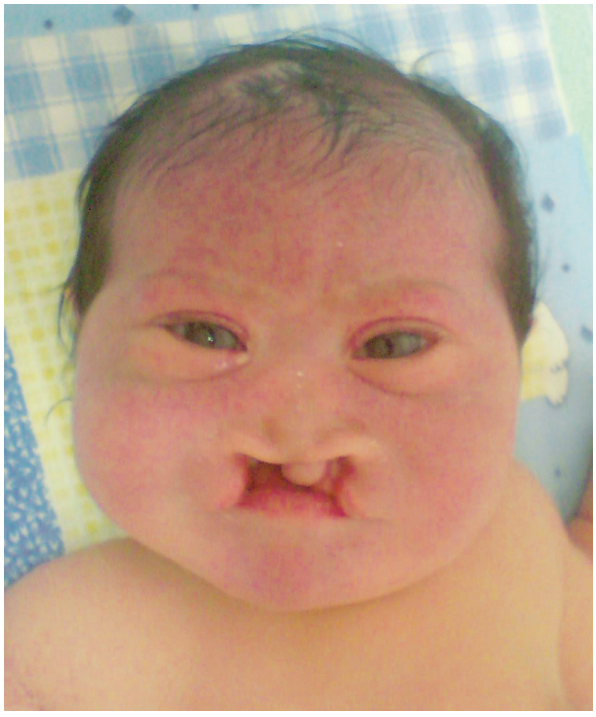

Figure 1. Facial view of the patient at birth showing hypertelorism with a large nasal bridge and a bilateral cleft lip and palate.

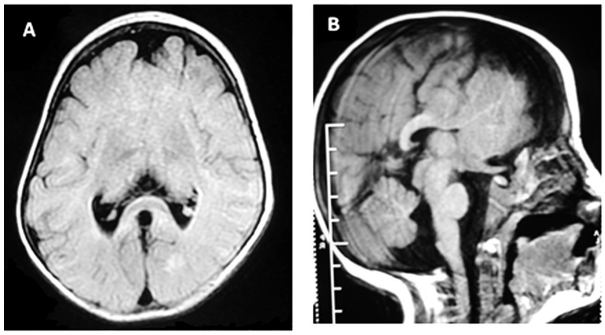

Figure 2. Brain MRI showing (A) holoprosencephaly and (B) callosal agenesis

Discussion

Mosaic trisomy 13, or mosaic Patau syndrome occurs when there is a portion of trisomic cells for an entire chromosome 13, while the remaining body cells are euploid.[7] The typical phenotype of complete trisomy 13 is usually associated with characteristic physical anomalies such as microcephaly, scalp defects, holoprosencephaly, microphthalmia, orofacial clefting, congenital heart defects, polydactyly, and profound mental retardation.[3] However, the phenotype of mosaic trisomy 13 varies widely, which renders clinical diagnosis difficult. Some patients may have the typical phenotype of trisomy 13 with neonatal death, while others may have few dysmorphic features and prolonged survival. The reason for this variation is that the phenotype changes according to the distribution of abnormal cells in specific tissues.[7] We clinically diagnosed our patient as having complete trisomy 13 by typical features, but chromosomal analysis revealed mosaic trisomy 13.

In the general population of children, the incidence of West syndrome ranges from 0.6 to 4.5 per 10,000 live births.[6,8] However, this incidence is much higher in children with Patau syndrome. It has been reported that 25 to 70% of patients with Patau syndrome have epilepsy, and 62.5% of these patients have epileptic spasms. They may present at an early age with myoclonic jerks and/or infantile spasms.[1,3,7] Spagnoli *et al.* reported that the age at onset of infantile spasms in 8 patients with trisomy 13 ranged from 2

months to 3 years and 9 months. West syndrome began during the first year of life in 50% of these patients.[1] Our patient had infantile spasms at 4 months.

The higher risk of West syndrome in Patau syndrome is shared with numerous other chromosomal abnormalities as in Down syndrome,[9] but the mechanism is not yet clear. According to the literature, two different hypotheses have been established.[1] The first hypothesis is based on the presence of malformations of cortical development in these conditions of mosaic trisomy 13. Malformations mainly affect midline development, with a high frequency of central nervous system involvement, including corpus callosum anomalies, ventriculomegaly, neural tube defects, hydrocephalus, holoprosencephaly, cerebellar dysplasia, olfactory aplasia, and cortical dysplasia. Interestingly, co-existing reflex and spontaneous seizures have been described in the setting of cortical malformations.[3,10,11] Brain MRI is recommended to facilitate the etiologic diagnosis of infantile spasms in patients with Patau syndrome.[4,6] In our patient, the brain MRI showed lobar holoprosencephaly and callosal agenesis. The second hypothesis involves genes on chromosome 13 for epilepsy and photosensitivity, and is supported by past research studies.1,12 Of interest is the recent indication of glypican-5 (GPC5) as a candidate gene for epilepsy inside the 13q31.[3] locus. Speculation has been made about a contribution of disrupted axon guidance and synaptic formation in the genesis of epilepsy and/or brain malformations in trisomy 13. However, at present, this hypothesis remains unproven.[1,12]

The diagnosis of West syndrome is often easy when infantile spasms are associated with arrest or regression of psychomotor development, and a specific EEG pattern of hypsarrhythmia.[8] The clinical symptoms of infantile spasms are very different from any other type of seizure because of the absence of paroxysmal motor phenomena, such as convulsions or loss of consciousness. This lack of typical seizure phenomena may lead to initial misdiagnosis of infantile spasms by pediatricians at the first medical consultation. Auvin **et al.** reported that approximately one-third of infants with infantile spasms were not suspected of having epilepsy during the first medical consultation.[13] Infantile spasms in infants are usually symmetrical and manifest as repetitive flexor, extensor, or flexor–extensor spasms, with sudden and brief axial contraction, predominantly in the upper limbs, with upper deviation of the eyes. In their cohort of 8 patients with Patau syndrome, Spagnoli et al. found a high prevalence of spasms and photic-induced myoclonic jerks and confirmed that photosensitivity manifested at an unusually early age of onset.[1] In our patient, West syndrome was clinically diagnosed from the flexor spasms that occurred several times a day, which began at an early age.

Medical treatment of infantile spasms should be effective and initiated as early as possible. Evaluation of treatment effectiveness includes cessation of spasms, resolution of hypsarrhythmia on the EEG, and reduction of the cognitive decline associated with epilepsy. Currently, vigabatrin and adrenocorticotropic hormone (ACTH) are the only drugs approved to suppress clinical spasms and abolish hypsarrhythmia. Past studies have reported different treatment protocols, but the large majority of children with infantile spasms received vigabatrin as first line treatment and ACTH as second line treatment.[6,14] Epileptic spasms in children with Patau syndrome were described as well-controlled with anticonvulsants.5 However, Spagnoli *et al.* presented evidence of difficult seizure control in two patients: one patient who needed multi-drug therapy and another patient who at clinical follow-up at 2 years of age experienced around 20 episodes of myoclonic seizures/day, despite high doses of sodium valproate. Neither of these patients underwent brain imaging.[1] In our patient, vigabatrin was immediately effective, with a complete resolution of clinical spasms during the first two years. The response to treatment has been shown to be significantly better when initiated less than 6 weeks after the diagnosis of infantile spasms.[15] These results suggest that early diagnosis and rapid treatment can improve longer-term prognosis of infantile spasms in children with Patau syndrome.

Despite early diagnosis and rapid initiation of effective treatment, West syndrome in children is still associated with a poor, long-term prognosis. Long-term follow-up showed that 60% of the children had drug-resistant epilepsy and that 75% had delayed psychomotor development.[16] Children with West syndrome and evidence of pre-existing developmental delay or neurological abnormalities, as in our patient, have a worse prognosis, poorer response to treatment, and less favorable developmental outcome.[6]

It is important to acknowledge that the prognosis of Patau syndrome is very poor and most patients die soon after birth because of severe congenital heart disease and brain anomalies. Almost 50% of cases die in the first month, and 90 to 95% of patients do not survive beyond one year of life. In the absence of severe cardiac and cerebral malformations, in particular the absence of holoprosencephaly, 5 to 10% of patients with trisomy 13 live longer than one year.[17,18] However, patients with mosaic trisomy 13 usually have longer survival and less severe phenotype compared to patients with complete trisomy 13. There have been several cases of long survival in patients with Patau syndrome, ranging from 3 to 38 years old.[1,3,10,18,19] Most of these survivors suffered from severe psychomotor delay, feeding difficulties, profound learning disability, seizures, as well as motor and mental deficits.[1,10,18] The patients who received intensive treatments survived longer and had better prognoses.[20,21] Although our patient had mosaic trisomy 13 syndrome, the control examination at 2 years of age showed severe developmental delay and tetraparesia.

The spectrum of phenotypic variations in mosaic trisomy 13 cases is broad. Therefore, genetic counseling of expecting parents with prenatally diagnosed mosaic trisomy 13 remains difficult. The majority of physical anomalies tend to be mild and non-life-threatening. Developmental delays and/or mental retardation, while being quite common, are not present universally. In all children with Patau syndrome, early developmental intervention and continued follow-up is essential for maximizing their cognitive skills.[10,22]

Patau syndrome patients are at increased risk of seizures. They may present at an early age with infantile spasms, but the spasms might not be easily controlled with antiepileptic medication.[1] This report describes the clinical characteristics and prognosis of a patient with mosaic trisomy 13 who survived for a relatively prolonged period and developed infantile spasms at an early age that were well-controlled with anticonvulsants.

Conflict of interest

None declared.

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Original Article

Habitual snoring and primary enuresis in children

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Abstract

Background Obstructive sleep-disordered breathing is assumed to be associated with primary enuresis in children. Prolonged enuresis may cause developmental and emotional disorders, as well as poor school performance.

Objective To determine the relationship between habitual snoring and primary enuresis in children.

Methods A cross-sectional study was conducted in Muara Batang Gadis District, North Sumatera in April 2016. Subjects were children aged 5-14 years. *The Sleep Disturbance Scale for Children (SDSC) Questionnaire* was used to measure the symptoms of sleep disordered breathing; the *International Association Child and Adolescent Psychiatry and Allied Professions (IACAPAP) Questionnaire* was used to assess for the presence of primary enuresis. The questionnaires were answered by the children's parents. Data were analyzed using Chi-square and logistic regression tests. A P value of <0.05 was considered to be statistically significant.

Results The mean age of 110 participants was 9.23 (SD 2.16) years. Twenty-seven (24.5%) subjects snored more than three nights per week (habitual snorers) and 18 (16.4%) subjects had primary enuresis. There was a significantly higher percentage of habitual snorers with enuresis than that of snorers without enuresis (55.5% *vs.* 18.4%, respectively) (P<0.05).

Conclusion There is a significant relationship between habitual snoring and primary enuresis. **[Paediatr Indones. 2018;58:116-22; doi: http://dx.doi.org/10.14238/pi58.3.2018.116-22]**.

Keywords: *: enuresis; habitual snoring; children*

Enuresis, which frequently occurs in children, is strongly related to sleep-disordered breathing (snoring).[1,2] Snoring therapy, with adenotonsillectomy or intranasal corticosteroid treatment, resulted in decreased incidence of enuresis in children.[3-5] Enuresis has long term effects if it is not well managed.[6] Enuresis may impact a child's development. Children may experience internal or external behavioral disorders, low self-esteem, poor grades in school, and even emotional disturbances.[7]

Habitual snoring is associated with enuresis. Snoring is the most common clinical manifestation occuring in children with obstructive sleep apnea (OSA).[8] Children with OSA do not have good quality sleep, resulting in decreased antidiuretic hormone (ADH) secretion. This drop in ADH can lead to micturition at night (nocturnal enuresis).[9]

Sex, age, low parental educational level, low

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socioeconomic status, and history of enuresis in the family are the variables which have been associated with enuresis and habitual snoring in children.[10] To date, enuresis and habitual snoring remain a problem for children in developing countries including Indonesia.

This study aimed to assess for a relationship between enuresis and habitual snoring in children.

Methods

This cross-sectional analytic study was conducted in the Muara Batang Gadis District, Mandailing Natal Regency, North Sumatera, in April 2016. The target population in this study was children aged 5-14 years; subjects were obtained using a simple, random sampling method. The exclusion criteria were urinary tract infection, spina bifida, cerebral palsy, or diabetes mellitus. Informed consent was obtained from subjects' parents or guardians. This study was approved by the Health Research Ethics Committee, University of Sumatera Utara Medical School.

Subjects underwent physical examinations and anthropometric measurements. Tonsil size was determined from direct inspection. The size was graded into 4 stage (T1-T4): T1 was normal size tonsil, T2 was hypertrophied tonsil with its medial edge reaching the midline of palatal arch, T3 was hypertrophied tonsil with kissing of both medial edges, and T4 was hypertrophied tonsil with no space between both medial edges. Subjects underwent urinalysis with urine rapid test to rule out urinary tract infection. Blood glucose level was measured with bedside rapid test (*Gluco dr*) to rule out hyperglycemia. The *SDSC Questionnaires*[11] to measure snoring were distributed to subjects and filled by their parents or sitters while the *IACAPAP Questionnaires*[1] were used to assess for the presence of primary enuresis. Demographic data were also obtained by interviews. All data were tabulated into a master table and appropriate statistical analyses were conducted.

The relationship between habitual snoring and enuresis was determined using Chi-square test. The risk factors for enuresis were analyzed using logistic regression test. The same test was used to determine the relationship between demographic factors and habitual snoring. Analyses were performed with statistical software *Statistical Package for Social Sciences (SPSS) version 15.0*. Results were considered to be significant for P values <0.05 with 95% confidence intervals.

Results

A total of 110 children were enrolled. The baseline characteristics of subjects are shown in **Table 1**. Subjects' mean age was 9.23 (SD 2.16) years. There were equal numbers of male and female subjects. On physical examination, 63 (57.3%) children had normal tonsils. No subjects had neurological, urinary tract, or metabolic disorders, nor did any have a history of medication usage which may affect diuresis.

All subjects lived with their parents, 27 (24.5%) had 3 siblings, and 26 (23.6%) lived with 6 other family members. Half of the subjects had siblings with history of enuresis and 54 (49.2%) had fathers with history of enuresis. The prevalence of obesity in this study was only 0.9% (1/110). Subjects were divided into groups: with and without enuresis, and with and without snoring. The prevalence of enuresis was 16.4% and the prevalence of snoring was 24.5% (**Table 2**).

Table 1. Baseline characteristics of subjects

Characteristics	(N=110)
Mean age (SD), years	9.23 (2.16)
Sex, n (%)	
Male	55 (50)
Female	55 (50)
Tonsils, n (%)	
T1-T1	63 (57.3)
T2-T2	22 (20.0)
T3-T3	25 (22.7)
History of enuresis in siblings, n (%)	
Yes	55 (50)
No	55 (50)
History of enuresis in fathers, n (%)	
Yes	54 (49.1)
No	56 (50.9)
History of enuresis in mothers, n (%)	
Yes	38 (34.5)
No	72 (65.5)
Obesity, n (%)	
Yes	1 (0.9)
No	109 (99.1)
Median number of house dweller (range), person	6.50 (3-13)

Table 2. Distribution frequency based on enuresis and snoring

Characteristics	(N=110)
Enuresis, n (%)	18 (16.4)
Yes	92 (83.6)
No	
Snoring, n (%)	
Yes	27 (24.5)
No	83 (75.5)

There were equal numbers of boys and girls in this study. In snoring group there was a domination of female (15/27) while in non-snoring group we found a male domination (51.8%) (**Table 3**). Tonsillar examination showed that 15/27 children in the snoring group had enlarged tonsils (T3-T3). In the non-snoring group, the majority of children (60/83, 72.3%) had normal tonsils. The history of enuresis in siblings, fathers, and mothers varied between the groups. In the snoring group, a history of enuresis in siblings and fathers was more common than a history of enuresis in mothers.

The non-enuresis group showed a different pattern. The majority of children had no enuresis history in siblings, fathers, or mothers. There were 2 obese children in the study, one in the snoring group and one in the non-enuresis group. In the enuresis group, 10/18 subjects were male, while the non-enuresis group was predominantly female (47/92, 51.1%). Normal tonsils (T1-T1) were observed in 7/18 of the enuresis group and 56/92 (60.9%) of the non-enuresis group. Histories of enuresis in fathers and mothers of the non-enuresis group were higher compared to those of the enuresis group (non-enuresis: 44/92, 47.8% and 28/92, 30.4%, respectively, vs. enuresis: both 10/18). The history of enuresis in siblings in the enuresis group was higher than in the non-enuresis group (13/18 *vs.* 42/92, 45.7%, respectively). There was no significant difference in obesity among subjects (**Table 4**).

Logistic regression test was conducted to analyze the risk factors of enuresis and snoring, including age, sex, obesity, number of siblings, history of enuresis in siblings, fathers and mothers, number of house dwellers, and tonsil size. History of enuresis in mothers and tonsil size were significantly associated with enuresis in children who snored (P=0.020 and 0.004, respectively). Of the two factors, large tonsil size was protective. The incidence of enuresis in subjects with snoring actually decreased 0.14 times with increasing tonsil size. Hence, the major risk factor for enuresis in the snoring group was history of enuresis in mothers, with an increased rate of

Table 3. Characteristics of subjects based on snoring and non-snoring

Characteristics	Snoring (n=27)	Non-snoring (n=83)	P value
Sex, n (%)			
Male	12	43 (51.8)	0.506
Female	15	40 (48.2)	
Tonsils, n (%)			
T1-T1	3	60 (72.3)	<0.001
T2-T2	9	13 (15.7)	
T3-T3	15	10 (12.0)	
History of enuresis in siblings, n (%)			
Yes	15	40 (48.2)	0.506
No	12	43 (51.8)	
History of enuresis in fathers, n (%)			
Yes	18	36 (43.4)	0.035
No	9	47 (56.5)	
History of enuresis in mothers, n (%)			
Yes	16	22 (26.5)	0.002
No	11	61 (73.5)	
Obesity, n (%)			
Yes	1	0 (0)	0.245
No	26	83 (75.5)	
Median number of house dweller (range), person	6 (4-13)	7 (3-11)	0.670

Table 4. Characteristics of subjects based on enuresis and non-enuresis

Characteristics	Enuresis (n=18)	Non-senuresis (n=92)	P value
Sex, n (%)			0.06
Male	10	45 (48.9)	
Female	8	47 (51.1)	
Tonsils, n (%)			0.150
T1-T1	7	56 (60.9)	
T2-T2	4	18 (19.6)	
T3-T3	7	18 (19.6)	
History of enuresis in siblings, n (%)			0.039
Yes	13	42 (45.7)	
No	5	50 (54.3)	
History of enuresis in fathers, n (%)			0.549
Yes	10	44 (47.8)	
No	8	48 (52.2)	
History of enuresis in mothers, n (%)			0.04
Yes	10	28 (30.4)	
No	8	64 (69.6)	
Obesity, n (%)			1.000
Yes	0	1 (1.1)	
No	18	92 (98.9)	
Median number of house dweller (range), person	6.5 (4-11)	6.5 (3-13)	0.954

enuresis of 18.132 times compared to the non-snoring group (**Table 5**).

Chi-square test revealed that snoring and enuresis in children had a statistically significant relationship (P=0.001) (**Table 6**).

Table 5. Risk factors analysis for enuresis in subjects with habitual snoring

Variables	Constant	Wald	P value*
Age	0.973	0.012	0.912
Sex	6.037	2.456	0.117
Number of siblings	1.751	0.510	0.475
History of enuresis in siblings	2.721	1.060	0.303
History of enuresis in fathers	0.117	2.879	0.900
History of enuresis in mothers	18.132	5.420	0.020
Number of house dwellers	0.459	0.833	0.361
Obesity	0.000	0.000	1,000
Tonsil size	0.140	8.488	0.004

Table 6. Relationship between habitual snoring and enuresis

	Enuresis	No enuresis	Total	P value*
Snoring	10	17	27	0.001
No snoring	8	75	83	
Total	18	92	110	

Discussion

Enuresis is a frequently neglected problem, mainly in children and adolescents. Several studies have recently shown that the prevalence of enuresis in children and adolescents is quite high. A previous study reported an enuresis prevalence of 25.9% in 4,203 children.[33] Another study reported a lower prevalence rate of enuresis in children in a rural area in India of 11.13%.[34] Another study conducted in Slovenia reported a prevalence of 12.8%.[35] A similar result was reported in Iran with prevalence rate of 11.01%.[36] The prevalence of enuresis in Indonesia from several studies was about 10.9%.[37] In our study, 18 of 110 children had enuresis, based on the *IACAPAP Questionnaire*.[1] The prevalence of enuresis was 16.4%, with 10 (9.1%) males and 8 (7.3%) females.

The risk factors of enuresis are socioeconomic, psychological, and genetic. Seventy-80% of children with enuresis had genetic disorders.[1] However, a previous study found that the risk factors of enuresis were socioeconomic status and the presence of urinary tract infection.[34] Similarly, another study found that psychological factors, socioeconomic level, and urinary tract infection were the risk factors of enuresis.[36]

Snoring is an important sign of an airway problem. This issue has caught much attention in developed countries, but not in Indonesia. Snoring can be classified into 2 types: habitual and non-habitual. The incidence of occasional snoring ranges from 26-30%, while habitual snoring is 5-7%. Habitual snoring needs to be managed because it can develop into obstructive sleep apnea syndrome (OSAS) and causes serious problems in children. The incidence of OSAS is approximately 0.1%-5.7% and increases with age, obesity, tonsil enlargement, and the other risk factors.[21] A previous study reported a prevalence rate of 27.3% for snoring in US children aged 6-18 years.[38] Similarly, our prevalence rate for snoring in children aged 5-14 years was 24.5%.

The relationship between habitual snoring and enuresis in children is still under debate. In a study of 42 children aged 3.5-14.5 years with sleep disturbances, as measured by polysomnography, the gold standard sleep quality assessment, 7 (16.7%) were found to have enuresis.[27] The authors concluded that habitual snoring and enuresis in children were significantly associated. In addition, Alexopoulos et al. reported that 135 (7.3%) of 1,821 children aged 5-14 years had habitual snoring and 7.4% of them had nocturnal enuresis. They, too, found that habitual snoring was associated with enuresis.[26] In our study, there was a significant relationship between habitual snoring and enuresis in children; 10/18 children who had habitual snoring also had enuresis. It was proven that there was a relationship between habitual snoring and enuresis in this study.

Snoring may cause a shallow state of sleep, leading to decreased antidiuretic hormone (ADH) secretion. Decreased ADH disrupts water retention in the bloodstream so that urine production increases. As such, this pathophysiology may explain how habitual snoring in children can cause enuresis. In our study there is a correlation between habitual snoring will cause enuresis in children.[20,26] There was a significantly higher percentage of habitual snorers with enuresis than that of snorers without enuresis (10/18 *vs.* 15.4%, respectively) ($P<0.05$).

A Sudan study of 290 children with tonsillar hypertrophy aimed to assess for relationships between tonsilar hypertrophy accompanied by snoring and/or enuresis; 114 (39.3%) of the subjects had enuresis. This finding led the authors to conclude that children with enuresis must be examined for tonsilar enlargement.[14] An Iranian study in 2013 was done to determine the prevalence of enuresis in children who had undergone tonsillectomy. A total of 420 children were enrolled, and 97 of them had a history of enuresis. Of these 97, only 84 agreed to participate in the study. Three months after tonsillectomy, 51 (60.7%) children no longer had enuresis and 22 (26.2%) children had improved enuresis symptoms ($P<0.001$). Enuresis did not improve in the remaining 11 subjects. Hence, the authors concluded that adenotonsillectomy can improve enuresis in children with adenotonsillar hypertrophy.[39]

The limitations of our study were its small sample size, small number of subjects with enuresis, using a questionnaire instead of polysomnography to assess snoring, and lack of data on tonsillectomies performed prior to the study.

In conclusion, there is a significant relationship between habitual snoring and enuresis in children. History of enuresis in mothers and small tonsil size are risk factors for enuresis in this study. In contrast, history of enuresis in fathers and siblings and tonsilar hypertrophy are not the risk factors for enuresis in snorers. There are significant differences in histories of enuresis in siblings and mothers between enuresis and non-enuresis groups. In the other hand, history of enuresis in fathers, obesity, and number of house dwellers are similar in both groups.

Conflict of Interest

None declared.

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Original Article

Correlation between non-exclusive breastfeeding and low birth weight to stunting in children

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Abstract

Background Indonesia is ranked fifth in the world for the problem of stunting. Stunting in children under the age of five requires special attention, due to its inhibiting effect on children's physical and mental development. Stunting has been associated with several factors, one of which is non-exclusive breastfeeding.

Objective To determine the correlations between non-exclusive breastfeeding and low birth weight to stunting in children aged 2-5 years.

Methods This case-control study was conducted in October to November 2016 in multiple integrated health service clinics (posyandu) in Sangkrah, Surakarta, Central Java, Indonesia. Subjects were children aged 24-59 months who visited the posyandu and were included by purposive sampling. Children classified as stunted were allocated to the case group, whereas the children classified with normal nutritional status were allocated to the control group. Parents filled questionnaires on history of exclusive breastfeeding, child's birth weight, maternal education, and family socio-economic status.

Results Of the 60 subjects, the control group had 30 normal children and the case group had 30 stunted children. Multivariate analysis by logistical regression test revealed statistically significant correlations between stunting and non-exclusive breastfeeding (adjusted OR for exclusive breastfeeding 0.234; 95%CI 0.061 to 0.894), as well as low birth weight (adjusted OR 10.510; 95%CI 1.180 to 93.572) This value implies that exclusive breastfeeding is a protecting factor against stunting, which means exclusive breastfeeding is able to decrease the prevalence of stunting in children under the age of five.

Conclusion In children aged 2-5 years, the histories of non-exclusive breastfeeding and low birth weights are significantly correlated sith stunting. **[Paediatr Indones. 2018;58:123-7; doi: http://dx.doi.org/10.14238/pi58.3.2018.123-7]**.

Keywords: *exclusive breastfeeding; stunting; children 24-59 months*

Stunting is a chronic nutritional deficiency caused by inadequate nutritional intake for an extended period, due to improper feeding. Chronic nutritional deficiency will influence the body length.[1,2] Stunting is defined as a Z-score of <-2 SD for body height to age ratio (BH/A) or body length to age ratio (BL/A), according to the World Health Organization (WHO) Child Growth Standard.[3] Indonesia is currently one of 117 countries worldwide with three highly prevalent nutritional problems in toddlers: stunting, wasting, and overweight, as reported in the *2014 Global Nutrition Report of Indonesia*.[4,5] The Indonesian Ministry of Health 2013 Basic Health Data (*Riskesdas*) reported a 37.2% prevalence of stunting in children under the age of five in Indonesia, which had increased compared to 2007 (36.8%), and 2010 (35.6%).[4]

Stunting in children under the age of five requires special attention due to inhibiting effects on physical and mental development. Stunting at early ages can increase the risk of mortality and morbidity, as well as suboptimal body posture as adults.[1] The WHO/UNICEF global strategy on

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feeding infants and young children recommends four important points to achieve optimal growth and development: early breastfeeding initiation (EBI) in the first 30 minutes of life, exclusive breastfeeding for the first 6 months of life, giving complementary food accompanied by breastfeeding at 6-24 months of age, and continuous breastfeeding for 2 years or more.[6,7] Growth and development during infancy requires balanced nutrition, as the toddler's intestinal system is still in the process of maturing. Breast milk contains numerous factors that meet the nutritional needs of infants according to their age.[8] Also, breast milk also contains immunological substances that can prevent infections in infants. Yet, the latest data have shown that exclusive breastfeeding behavior in the first 6 months of life remains inappropriately with the recommendation.[5-7] *The 2015 WHO/UNICEF Data on Infant and Young Child Feeding* showed that only 39% of babies in developing countries received exclusive breastfeeding from 0-5 months.[6] Hence, we aimed to determine the correlation between non-exclusive breastfeeding and the incidence of stunting in children aged 2-5 years.

Methods

This observational analytic study with a case-control approach was done in October – November 2016 to analyze for a relationship between a history of exclusive breastfeeding and stunting in children aged 2-5 years. Subjects were patients aged 24-59 months at multiple integrated health service clinics (posyandu) under the auspices of the primary health care integrated service unit in Sangkrah, Surakarta, Central Java, Indonesia. Children's nutritional status was assessed by way of body height/age (BH/A) index. Stunted children were allocated to the case group and children without stunting were allocated to the control group. Subjects' parents provided written informed consent. Children with infection or other chronic conditions at the time of the study were excluded.

Sixty children were included by purposive sampling, to which the rule of thumb was applied, resulting in 30 subjects per group.[9] Data on exclusive breastfeeding, birth weight, maternal education, and socio-economic status based on Surakarta's minimum wage were collected using questionnaires. Low socio-economic level was defined as family income of less than the minimum regional wage of Surakarta in 2016, which was rounded up to Rp 1,400,000. Education level was defined as the length of education less than 9 years and higher than 9 years. Body height was measured three times, using a microtoise with 0.1cm accuracy, and mean values were calculated. The measurements were conducted on the same day or within three days at most of the interview.

The dependent variable of this study was stunted nutritional status, while the independent variable was the history of exclusive breastfeeding. External variables were the maternal educational status, socio-economic status, and history of low birth weight.

Chi-square test was used for bivariate analysis and logistical regression test was used for multivariate analysis, using statistical product and service solution (*SPSS) 24.0* for *Mac*, with significance of $P<0.05$. The Research Ethics Committee of Sebelas Maret University Medical School, Surakarta, approved this case control study.

Results

Table 1 shows that the case group had a rather balanced sex ratio, with 15 children of each sex. The control group consisted of 14 boys (46.7%) and 16 girls (53.7%). The independent variable of exclusive breastfeeding was differentiated into exclusively breastfed or not exclusively breastfed. In the case group, 17 children (56.7%) were exclusively breastfed, while 13 children (43.3%) were not exclusively breastfed in other words, they receved infant formula or complementary foods. In the control group, 26 children (86.7%) were exclusively breastfed, and 4 children (13.3%) were not exclusively breastfed. There were 6 children in the case group and one in the control group who did not receive breast milk the first day of life.

Low birth weight was defined as birth weight < 2500 grams. The case group had 9 children (30%) with a history of low birth weight and 21 children (70%) with normal birth weight. In the control group, 1 child (3.3%) had a history of low birth weight, while 29 children (96.7%) had normal birth weight.

Maternal education was classified as either low (less than 9 years) or high (more than 9 years). In the case group, 10 subjects (33.3%) had low, while

20 (66.7%) had high maternal educational status. In the control group, 14 subjects (46.7%) had low and 16 (53.3%) had high maternal educational status.

Socio-economic status was classified as low (< IDR 1400000 monthly income) or high (> IDR 1400000 monthly income). In the case group, 21 children (70.0%) had low and 9 children (30.0%) had high socio-economic status. In the control group, 24 children (83.3%) had low and 6 children (16.6%) had high socio-economic status. Overall, 45 children (75%) had parents with low socio-economic status and 15 children (25.5%) had parents with high socio-economic status (**Table 1**).

Table 1. Characteristics of subjects

Characteristics	Case group (n=30)	Control group (n=30)
Sex, n (%)		
Male	15 (50.0)	14 (46.7)
Female	15 (50.0)	16 (53.3)
Exclusive breastfeeding, n (%)		
Yes	17 (56.7)	26 (86.7)
No	13 (43.3)	4 (13.3)
Birth weight, n (%)		
Low	9 (30.0)	1 (3.3)
Normal	21 (70.0)	29 (96.7)
Maternal education, n (%)		
Low	10 (33.3)	14 (46.7)
High	20 (66.7)	16 (53.3)
Socio-economic status, n (%)		
Low	21 (70.0)	24 (83.3)
High	9 (30.0)	6 (16.6)

Table 2 shows the bivariate statistical analysis results. Chi-square test revealed that stunting had significant correlations with non-exclusive breastfeeding (OR for exclusive breastfeeding: 0.201; P=0.010) and low birth weight (OR 12.429; P=0.006). There were no significant correlations between stunting and sex (P=0.796), maternal education (P=0.292), or socio-economic status (P=0.371).

Multivariate analysis by logistical regression test similarly revealed statistically significant correlations between stunting and non-exclusive breastfeeding (adjusted OR for exclusive breastfeeding 0.234; P=0.034), as well as low birth weight (adjusted OR 10.510; P=0.035) (**Table 3**).

Table 3. Multivariate analysis

Variables	Adj OR	P value	95%CI
Eclusive breastfeeding	0.234	0.034	0.061 to 0.894
Low birth weight	10.510	0.035	1.180 to 93.572

Discussion

There was a significant relationship between non-exclusive breastfeeding and stunting in children aged 24-59 months. More children with normal nutritional status received exclusive breastfeeding (86.7%) than stunted children (56.7%). Bivariate analysis showed that exclusive breastfeeding was a protective factor against stunting, with OR 0.201. Furthermore, multivariate analysis revealed that exclusive breastfeeding was still a protective factor, with OR 0.234 (95%CI 0.061 to 0.894). A 2010 study in Banda Aceh similarly reported that stunting in children under the age of five was associated with non-exclusive breastfeeding, with a 5 times higher risk of stunting than children under five who had received exclusive breastfeeding.[10]

According to interviews, most mothers gave their children formula in addition to breast milk. Their reasons variations in breast milk production, infant lack of appetite for breast milk, and maternal work outside the home. Feeding of formula and breast milk can satisfy the nutritional requirements of the child, but formula lacks antibodies. As such, the child would be prone to diseases.[11] Breast milk contains numerous immunological substances not found in formula, such as immunoglobins that can prevent disease, secretory substances that can

Table 2. Bivariate statistical analysis

Variables	OR	95%CI	P value
Sex	0.875	0.318 to 2.410	0.796
Exclusive breastfeeding	0.201	0.056 to 0.721	0.010
Maternal education	1.750	0.616 to 4.97	0.292
Low birth weight	12.429	1.461 to 105.737	0.006
Socio-economic status	1.714	0.371 to 5.621	0.523

neutralize pathogenic *E. coli* and multiple viruses of the digestive track, as well as lactoferrin, an immunological substance that binds iron from the digestive track and has bactericidal properties.[12] Breast milk also contains a 65:35 ratio of whey to casein, while formula has a ratio of 20:80. As such, proteins and other substances in breast milk tend to be more easily absorbed compared to formula milk.[12]

More children with history of low birth weight were in the case group than in the control group. We found that history of low birth weight increased the risk of stunting more than 12 times compared to those with normal birth weight. A Zimbabwe study also found that more infants with history of low birth weight (41.4%) experienced stunting.[13] The effect of birth weight on stunting happens in the first 6 months of life, then decreases until the age of 24 months. As such, if infants can catch up in their growth in the first 6 months of life, there is a higher chance for them to achieve normal body height.[14] Infants with history of low birth weight have been shown to have growth retardation in utero, both acutely and chronically. Hence, these children are prone to infections such as diarrhea and lower respiratory tract infections, as well as increased probability of icterus, anemia, chronic lung problems, exhaustion, and loss of appetite compared to children with normal birth weight.[15] In our study, 70% of those in the case group had a history of normal birth weight. This may have been due to inadequate nutrition in these otherwise normal infants, which caused growth faltering (failure to thrive).[16]

We found no significant relationship between socio-economic status and stunting (P=0.371). Low socio-economic status was noted in 70% of the case group, and 83.3% of the control group. In contrast, a study in the North Moluccas reported a significant relationship between stunting/severe stunting and low socio-economic status in children aged 0-59 months.[17] The food consumed by low income families is less varied and lower in quantity, particularly in terms of nutrition required for childhood growth, such as sources of protein, minerals, and vitamins. As such, there is a higher risk of malnutrition in these children.[18] The contrasting results in our study may have been due to different socio-economic measurements.

Low parental education level, of both fathers and mothers, may increase the risk of stunting, but we did not find this to be the case in our study. We found no significant relationship between stunting and maternal educational level (P=0.292). Similarly, Nasikhah (2012) in East Semarang reported that bivariate and multivariate analyses revealed no statistical significance between stunting and maternal educational level.[19] This finding may have been due to the mothers with high educational status giving their infants formula, because they work outside home and cannot breastfeed their children. Working mothers have less time for their children, perhaps leading to malnutrition in a later stage and influencing growth and development.[20]

In conclusion, there is a significant relationship between non-exclusive breastfeeding and stunting. Breastfeeding may be a protective factor against stunting in children under the age of five. Low birth weight infant also have a significant relationship with stunting. Further study should include not yet investigated variables, a larger sample size, and larger population coverage.

In light of our findings, we suggest that health workers, promote breastfeeding to mothers during pregnancy and after delivery. Mothers should be informed of the recommended practice of breastfeeding from childbirth to the age of 6 months, and about factors affecting the nutritional status of children aged 24-59 months, specifically to prevent stunting. In addition, expecting and new mothers should educate themselves on the nutritional requirements of infants, and early detection of stunting, so that they can lower the risk in their toddlers.

Conflict of Interest

None declared.

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Original Article

Serum IGF-1 and short stature in adolescents with ß-thalassemia major

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Abstract

Background The prevalence of short stature in thalassemia patients ranges from 39.3 to 65%. The cause of short stature is complex and still up for debate. In Indonesia, data on the prevalence and risk factors of short stature in adolescents with thalassemia have been limited.

Objective To assess for the prevalence and risk factors of short stature in adolescents with beta-thalassemia major.

Methods This cross-sectional study was done from February to March 2017 at the Thalassemia Clinic at Dr. Hasan Sadikin General Hospital, Bandung, West Java. The baseline characteristics data of 80 adolescents with thalassemia aged 10-14 years were recorded. Short stature was assessed by height-for-age, (Z-score <-2SD) based on the *2007 WHO Reference Growth Chart*. Mid-upper arm circumference was scored according to age and sex and serum IGF-1 was measured by ELISA method.

Results Subjects were 40 males and 40 females, 81.2% of whom had short stature. The mean serum IGF-1 level was 32.2 (SD 26.38) ng/mL. The IGF-1 cut-off point by ROC curve was ≤ 38.51 ng/mL, with sensitivity of 64.4% and specificity of 86.7%. The risk factors of short stature were IGF-1 level ≤ 38.51 ng/mL (PR 40.66; 95%CI 4.37 to 377.58) and low family income (PR 19.76; 95%CI: 1.152 to 256.08).

Conclusion IGF-1 level may be useful as a predictor of short stature in adolescent beta-thalassemia major patients. **[Paediatr Indones. 2018;58:151-8; doi: http://dx.doi.org/10.14238/pi58.4.2018.151-8]**.

Keywords: *adolescent; IGF-1; short stature; thalassemia*

About 39.3 to 65% of children with thalassemia have growth disorders during the fetal, neonatal, pre-pubertal, and pubertal periods.[1-5] About 20-30% might have growth hormone deficiency and 80% might have very low growth hormone that were lower than would be expected for constitutional short stature.[6] In Indonesian thalassemia patients, 65% had short stature, 20% delayed puberty, 41% hypoparathyroidism, and 29% delayed bone age.[5] A previous study at Dr. Hasan Sadikin Hospital found that 67% of 10 to14-year-old patients had delayed growth.[7] Another study reported that more than half of thalassemia patients aged 13.8 and >15 years had delayed growth. The delayed growth and puberty might also occur in 12 to 17-year-old thalassemia patients.[8]

The cause of growth disorders on thalassemia patients is complex and still being debated. The growth hormone (GH) and insulin-like growth factor-1 (IGF-1) axis was reported to have an

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important role in these patients. The most probable cause of growth disorders is the decrease in serum IGF-1 concentration in response to GH.[9] We aimed to identify the risk factors of short stature in thalassemia patients, in hopes that adolescents with thalassemia who have undergone proper treatment and management including routine transfusion, iron chelation, and monitoring of side effects, can achieve optimal growth according to age for a longer and better quality of life.

Methods

Eighty patients with thalassemia major were recruited at the Thalassemia Clinic, Dr. Hasan Sadikin General Hospital, West Java, Indonesia, during February-March 2017. Subjects' parents provided informed consent after having been briefed on the study protocol.

The inclusion criteria were children aged 10-14 years, diagnosed with beta-thalassema major, and who had undergone routine transfusions. The exclusion criteria were patients with other chronic diseases (malignancy, tuberculosis, chronic hepatitis, congenital heart disease, chronic kidney injury, epilepsy, or diabetes mellitus), another type of thalassemia, nutritional disorders, or congenital syndromes, other than family history of short stature.

For this cross-sectional study we collected subjects by consecutive sampling of thalassemic adolescents who fulfilled the inclusion criteria and routinely visited the Thalassemia Clinic at Dr. Hasan Sadikin Hospital. We recorded patient data and interviewed parents about the time of diagnosis, the use of iron chelation and number of transfusions, and subjects provided blood specimens for IGF-1 hormone examination. We performed physical and anthropometric examinations [body weight, height, and mid-upper arm circumference (MUAC)]. The hemoglobin (Hb) level before and after transfusion, and the ferritin level within the three months prior were collected from the medical records or the records from the Clinical Pathology Laboratory of Dr. Hasan Sadikin Hospital, Bandung.

Body weight was measured by *SECA sensa 804* scale, with 0.1 kg accuracy. Body height examination using microtoise *SECA 217*. Short stature was assessed by height-for-age, (Z-score <-2SD) and body weight-for-age basaed on *2007 WHO Reference Growth Chart*.[10] For nutritional assessment, the MUAC measurement was taken midway between the tip of the acromion and olecranon processes, and classified according to Frisancho as follows: below adequate: ≤ 5th percentile; adequate: between 5th and 95th percentile; and above adequate: ≥95th percentile.[11,12] Sexual maturation was assessed by Tanner criteria.[13] Testicular volume was evaluated by Prader orchidometer.[14]

Serum IGF-1 concentration was measured by enzyme-linked immunosorbent assay (ELISA)-*Mediagnost®*. The mean inter- and intra-assay coefficient of variation (CV) was determined to be 6.8% and 6.7%. The mean minimum detectable concentration of IGF-1 in this assay was 0.09 ng/mL. with classified: very low levels, i.e below the age-related 0.1th percentile, low levels, i.e. close to or below the age-related 5th percentile and normal level, i.e. above the age-related 5th percentile. The IGF-1 levels were measured at Department Clinical Pathology and Laboratory, Dr. Hasan Sadikin Hospital, Bandung.

Chi-square, Mann-Whitney, or Fisher's exact tests were used to assess for associations between characteristics and short stature. Logistic regression analysis was used to determine the most significant factors associated with short stature. A P value of <0.05 was considered to be statistically significant. The study was approved by the Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital, Bandung.

Results

This study on the risk factors of short stature in adolescents with beta-thalassemia major was performed at the Thalassemia Clinic, Dr. Hasan Sadikin Hospital, Bandung. During February-March 2017, 102 patients aged 10-14 years visited the Thalassemia Clinic. We interviewed parents and performed anthropometric examinations (weight, height, and mid-upper arm circumference), maturation assessment, and blood sampling on the patients. Eighty patients fulfilled the inclusion criteria and were analyzed, as shown in **Figure 1**.

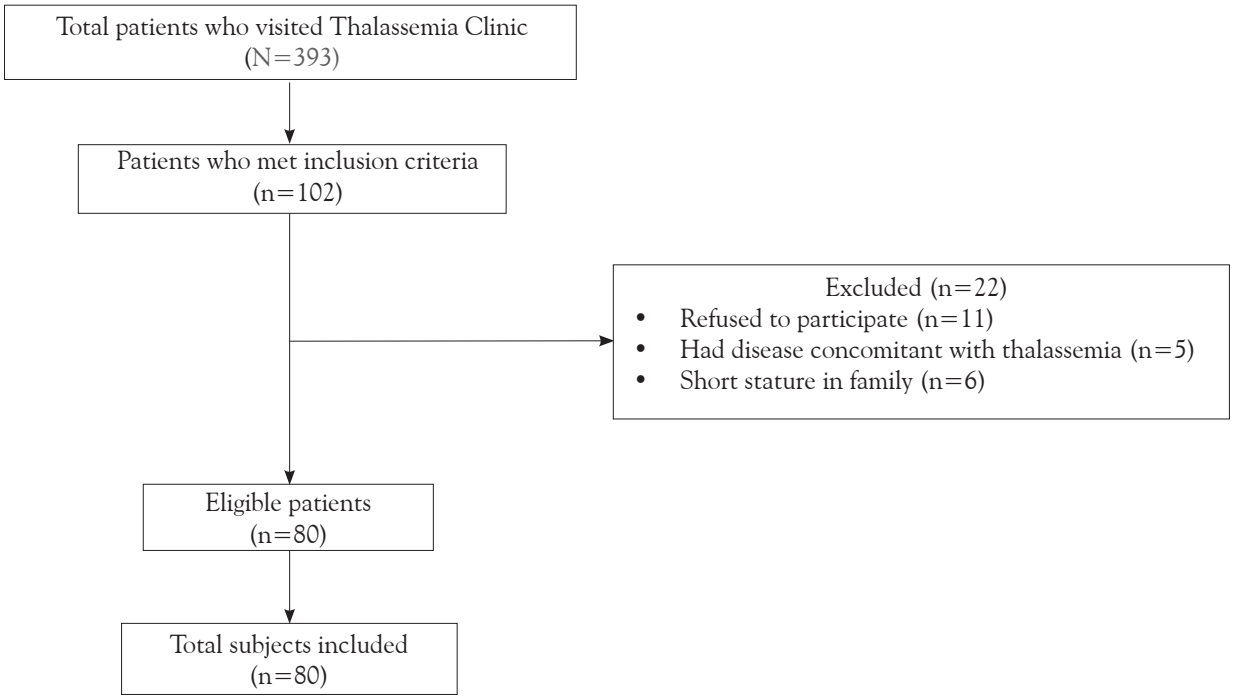

Figure 1. Study flow chart

Table 1 shows that the numbers of male and female subjects were the same. Based on the MUAC by age and gender, nutritional status of subjects was as follows: 47 (58.8%) subjects below adequate, 33 (41.2%) had adequate, and none had above adequate nutrition. Short stature (height-for-age Z-score <-2SD) was observed in 65 (81.2%) subjects. For IGF-1 concentrations, 76 (95%) subjects had very low IGF-1 levels and only 1 (1.3%) subject had a normal level.

Table 2 shows the comparison of various characteristics between the short stature and normal height groups. There was a significant difference in mean IGF-1 levels between short stature and normal subjects [32.22 (SD 26.38) *vs.* 68.58 (SD 51.46) ng/mL, respectively (P<0.001)]. The median IGF-1 level in the short stature group was also significantly lower than that of the normal group [30.94 (range 1.03-150.72) *vs.* 49.54 (range 14.87-187.68) ng/mL, respectively (P <0.001)].

Since IGF-1 levels were significantly lower in the short stature group than in the normal height group, a receiver-operator characteristic (ROC) curve analysis was done to define an IGF-1 cut-off level, as a predictor for short stature. The ROC curve revealed the IGF-1 cut-off point to be ≤38.51 ng/mL, as a predictor of short stature. Furthermore, using the IGF-1 cut-off ≤ 38.51 ng/mL, we analyzed for a correlation between serum IGF-1 and short stature, as shown in **Table 3**. The IGF-1 cut-off point of ≤ 38.51 ng/mL had 64.6% sensitivity, 86.7% specitivity, and 68.8% accuracy. Adolescents with beta-thalassemia major and IGF-1 level ≤ 38.51 ng/mL had 1.49 times higher risk of short stature compared to those with IGF-1 > 38.51 ng/mL. As such, IGF-1 may be useful to predict short stature in beta-thalassemia major patients of adolescent age.

Multiple logistic regression analysis was used to further analyze bivariate results with P<0.25, and clinical significance. The results are shown **Table 4**. Adolescents with beta-thalassemia and low family income had 19.8 times the risk of short stature (PR19.76; 95%CI 1.52 to 256.08; P=0.022) and IGF-1 level ≤38.51 ng/mL had 40.7 times the risk of short stature (PR40.66; 95%CI 4.37 to 377.58; P<0.001).

Table 1. Baseline characteristics of subjects

Characteristics	N=80
Median age (range), months	140 (120-200)
Sex, n(%)	
Male	40 (50)
Female	40 (50)
Family history of thalassemia, n(%)	
Yes	14 (17.5)
No	66 (82.5)
Family income, n(%)	
Low (< IDR 1,500,000)	32 (40)
High (>IDR 1,500,000)	48 (60)
Nutritional status (MUAC for age and sex), n(%)	
Below adequate	47 (58.8)
Adequate	33 (41.2)
Height-for-age (Z-score), n(%)	
Normal	15 (18.8)
Stunted	27 (33.8)
Severely stunted	38 (47.4)
Puberty, n(%)	
Appropriate	80 (100)
Age at the time of diagnosed, n(%)	
<6 years	75 (93.7)
≥6 years	5 (6.3)
Iron chelation therapy, n(%)	
Yes	77 (96.3)
No	3 (3.7)
Type of iron chelation therapy, n(%)	
Deferoxamine/DFO	1 (1.3)
Deferasirox/DFX	27 (33.7)
Deferiprone	49 (61.2)
None	3 (3.8)
Serum ferritin level, n(%)	
<2,000 ng/mL	18 (22.5)
≥2,000 ng/mL	62 (7.5)
Duration of time since the diagnosis, n(%)	
<8 years	10 (12.5)
≥8 years	70 (87.5)
Pre-transfusion hemoglobin level, n(%)	
<9 g/dL	80 (100)
Number of transfusions per month, n(%)	
<2x	78 (97.5)
3x	1 (1.3)
4x	1 (1.3)
IGF-1 levels, n(%)	
Very low	76 (95)
Low	3 (3.7)
Normal	1 (1.3)
History of chemical agent, n(%)	
Yes	9 (11.3)
No	71 (86.7)

Discussion

This study was conducted to assess for risk factors of short stature in adolescents with beta-thalassemia major. There were 80 subjects aged 10-14 years, consisting of 40 (50%) males and 40 (50%) females. Nutritional status was assessed by MUAC, according to age and sex. Below adequate nutritional status was seen in 47 (58.8%) of subjects, adequate in 33 (41.2%) subjects, and above adequate in none of the patients. About 65 (81.2%) of the subjects had short stature (stunted and severely stunted, based on height-for-age Z-score).

Hashemi *et al.* reported that 65.71% of thalassemia patients had short stature.[15] Similarly, another study at the Thalassemia Clinic of Dr. Hasan Sadikin Hospital, Bandung on thalassemia patients aged 10-14 years found that 62% had short stature.[7] Both studies assessed nutritional status using body mass index for age, with Hashemi *et al.* reporting 81.4% normal and 18.6% malnourished subjects.[15] Rachmat *et al.* assessed nutritional status of thalassemia patients by upper arm circumference for age, and found that 50.9% had normal nutritional status and 49.1% had malnutrition.[16] Many factors may contribute to short stature in thalassemia patients, such as nutritional deficiency, chronic anemia, hypersplenism, zinc deficiency, growth hormone deficiency, and disorders of the hypothalamus-hypophysis gonadal axis.[17] Of those with beta-thalassemia major, 57.6% had disorders of linear growth and 45.5% had pubertal disorders noted as adults.[18]

Thalassemia patients generally receive combined therapy of blood transfusion and chelation.[19-21] Routine blood transfusion might result in better prognosis, but can lead to iron accumulation that disturbs cellular processes.[22] In our study, there was no significant difference in Hb level before blood tranfusion between the short stature (6.79 g/dL) and normal (6.76 g/dL) groups (P=0.566). Similarly, Pemde *et al.* found no correlation of body height Z-score and mean Hb pre-transfusion.[23] At present, patients with beta-thalassemia major typically have Hb levels of above 9 g/dL or 9.5-10 g/dL,[19,22,24] to prevent bone abnormality and splenomegaly.[22] Hb levels of below 9 g/dL is caused by a lack of compliance to receive regular blood transfusions. Also, low family income may affect the compliance.

Table 2. A comparison of characteristics between the short stature and normal height groups

CharacteristicS	Short stature (n=65)	Normal height-for-age (n=15)	P value
Age, months			
Mean (SD)	144.12 (15.9)	140.53 (16.6)	0.378*
Median	140	135	
Range	120-200	123-170	
Sex			
Male	33 (50.8)	7	0.775**
Female	32 (49.2)	8	
Family income			
Low (<IDR 1,500,000)	29 (44.6)	3	0.229**
High(>IDR1,500,000)	36 (55.4)	12	
Time of diagnosed			
<8 years	9 (13.8)	1	0.678***
≥8 years	56 (86.2)	14	
Pre-transfusion hemoglobin level (g/dL)			
Mean (SD)	6.79 (1.05)	6.76 (1.13)	0.566*
Median	6.60	7.00	
Range	4.60-9.10	5.70-8.10	
Total transfusion per month			
<2 x	63 (83.1)	13 (16.9)	1.000***
>3 x	2 (33.3)	2 (66.7)	
Median	3,900	4,200	
Range	2,700-5,700	3,000-5,700	
Iron chelation therapy			
Yes	64 (98.5)	13 (16.9)	0.089***
No	1 (1.5)	2 (66.7)	
Serum ferritin level, ng/mL			
Mean (SD)	3,704.54 (1,998.48)	3,775.91 (1,993.40)	0.510*
Median	3,824	3,450	
Range	775-10,223	721-7,327	
Type of iron chelation therapy			
Deferoxamine/DFO	1 (1.5)	0	0.118**
Deferasirox/DFX	24 (36.9)	3	
Deferiprone	39 (60.0)	10	
None	1 (1.5)	2	
IGF-1 value, ng/mL			
Mean (SD)	32.22 (26.38)	68.58 (51.46)	<0.001*
Median	30.94	49.54	
Range	1.03-150.72	14.87-187.68	
Nutritional status			
Below adequate	40 (61.5)	7	0.292**
Adequate	25 (38.5)	8	

SD = standard deviation; *Mann-Whitney test; * *Chi-square test; *** Fisher's exact test

Table 3. Correlation between serum IGF-1 concentration and short stature

	IGF-1 level (ng/mL)	Short stature, n(%) (n=65)	Normal, n(%) (n=15)	PR (95%CI)	P value
Cut-off point	≤38.51	42 (64.6)	2	1.49 (1.16 to1.93)	<0.001
	>38.51	23 (35.4)	13		

PR: prevalence ratio (95% confidence interval); sensitivity: 42/65=64.6%; specificity: 13/15 = 86.7%; positive predictive value: 42/(42+2) = 95.5%; negative predictive value: 13/(23+13)=36.1%; accuracy: (42+13)/80=68.8%.

Table 4. Multivariate analysis between the variables and short stature

Variables	Koev (B)	SE (B)	PR (95%CI)	P value
Serum ferritin level	-8.178	3.908	0.64 (0.08 to 4.78)	0.660
Family income				
Low	2.984	1.307	19.76 (1.52 to 256.08)	0.022*
High	0.907	1.128	2.52 (0.28 to 23.02)	0.412
Pre-transfusion Hb	0.856	0.486	2.35 (0.91 to 6.10)	0.078
Iron chelation therapy	1.681	1.671	5.37 (0.20 to 141.85)	0.314
Nutritional status	0.491	0.756	1.63 (0.37 to 7.19)	0.516
IGF-1 value ≤38.51 ng/mL	3.704	1.138	40.66 (4.37 to 377.58)	0.001*

Note: *significant if P<0.05; logistic regression analysis

High levels of ferritin have been strongly correlated with growth disorders, endocrine disorders, or other complications.[20] As such, chelation therapy may prevent these complications.[24] Serum ferritin measured at routine intervals (at least every 3 months) and can be used as proxy measures, with recommended target levels of 1,000 ng/mL.[19,20] We found no significant difference in the range of serum ferritin level between the short stature (775-10,223 ng/mL) and normal height (721-7,327 ng/mL) groups (P=0.510). In contrast, Joshi *et al.* reported the range of serum ferritin level of 5,295 (SD 2,736) ng/mL and Hb value of 7.8 (SD 0.6) g/dL were not significantly different in the thalassemia group and normal group.[25] Another study found a correlation between serum ferritin level >2,000 ng/mL and height-for-age (Z-score) >10-15 years (P<0.001).[23] Study in Indonesia reported a correlation between serum ferritin level (OR=3.248; 95%CI 1.304 to 8.086) and growth disorder in thalassemia patients (OR=3.964; 95%CI 1.192 to 13.190).[26]

Iron chelation therapy is used to prevent increased ferritin level and subsequent organ injury.[27] Three kinds of chelation therapy generally used are deferoxamine (DFO), deferiprone (DFP), and deferasirox (DFX). Iron chelation is usually started after 10-20 blood transfusions or if the serum ferritin level is more than 1,000 ng/mL. Deferasirox is more commonly used because of its lower toxicity,[28] but several studies have found a high incidence of short stature in thalassemic children and adolescents who received DFX therapy.[24] We found no significant difference between the short stature and normal groups among the three chelation therapy types (P=0.118). A number of 96.2% got deferiprone as iron chelation therapy and there was no significant relation towards short stature on thalassemia patients (P=0.089 and P=0.118). More than 50% of our subjects had chelation therapy, but how optimal the use of iron chelation therapy is unknown.

In our study, subjects with low family income had 19.7 times increased risk of short stature (PR 19.76; 95%CI 1.52 to 256.08; P=0.022). In addition, subjects with serum IGF-1 ≤ 38.51 ng/mL had 40.7 times increased risk of short stature (PR 40.66; 95%CI 4.37 to 377.58; P=0.001). Other factors such as long transfusion duration, chelation therapy, ferritin level, duration of time since the diagnosis , Hb level, number of transfusions, and nutritional status were not significant risk factors for short stature. Another cross-sectional study on thalassemic patients revealed that patient age (OR 5.42; 95%CI 1.29 to 12.41; P=0.016) and low family income (OR 2.32; 95%CI 1.06 to 5.06; P=0.036) were risk factors of growth disorders.[18] Yet another study reported that age at diagnosis, number of transfusions, size of lymph, serum ferritin level, and Hb level were associated with growth disorders in thalassemic patients (P<0.05).[2] Al-Salehe *et al.* found that short stature in thalassemic patients was strongly associated with high serum ferritin (P=0.006), but was not associated with sex, history of splenectomy, more than one tranfusion per month, or the use of chelation therapy (deferasirox and desferoxamine).[29]

The growth orders of the IGF-1, the IGF binding protein-3 (IGFBP3), and acid-labile subunit (ALS), all these proteins are mentioned to be the biomarker of secretion, activities and physiologic role of GH.[30] The IGF-1 produced by the liver[31] is an important growth factor on most of the growth effects.[30,32] The serum

level of IGF-1 is affected by nutritional status and other hormones, such as insulin.[32] Deficiency of circulating IGF-1 can lead to malnutrition, hypothyreosis, as well as renal and liver insufficiency.[31] In thalassemia major, IGF-1 deficiency has been attributed to chronic anemia, hypoxia, chronic liver disease, iron overload, and other associated endocrinopathies, e.g., GH deficiency.[33] Other studies have noted decreased IGF-1 levels in thalassemia patients,[18,31,32] similar to our results in that all subjects had decreased IGF-1 levels, except for one subject with normal serum IGF-1 level.

Using IGF-1 as an indicator of GH deficiency is still debatable, as it has low specificity. However, while low IGF-1 could strengthen a diagnosis of GH deficiency, GH stimulation still needs to be performed as the gold standard of GH deficiency, especially when the IGF-1 level is normal ($r=0.56$ and $P>0.05$).[31] The correlation of IGF-1, IGFBP-3, and body height-for-age remains unclear in thalassemic patients, indicating that growth disorders may be related, not only to the GH-IGF-1 axis.[24] Ali *et al.* reported an IGF-1 diagnostic sensitivity of 83.87% and specificity of 76.2%.[34] However, Alawneh *et al.* found a 47% sensitivity and 65% specificity in detecting GH deficiency.[31]

A limitation of our study was not performing the growth hormone stimulation test, nor assessing for a correlation between IGF-1 and GH. So far, there is no study which reveal about the sensitivity and specificity of IGF-1 level based on short stature on thalassemia patients. In our study, we found that patients with IGF-1 value ≤38.51 ng/mL had 1.49 times higher risk of short stature compared to those with IGF-1 value >38.51 ng/mL, with sensitivity of 64.6%, specificity of 86.7%, and accuracy of 68.8%. Based on these results, IGF-1 level could be useful in identifying children with beta-thalassemia major who are at risk of short stature. Another limitation of this study was analyzing serum ferritin levels instead of the actual serum ferritin levels at the exact assessment time of growth status, we used serum ferritin levels in the 3 months prior and need for growth monitoring, nutrition and medication adherence from early diagnosis of beta thalassemia major. In conclusion, serum IGF-1 level of ≤38.51 ng/mL is a risk factor for short stature in adolescents with beta-thalassemia major.

Conflict of interest

None declared.

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Original Article

Liver function in children with human immunodeficiency virus infection before and after 6 months of highly active antiretroviral therapy

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Abstract

Background Highly active antiretroviral therapy (HAART) has resulted in dramatic decreases in morbidity and improved survival rate in human immunodeficiency virus (HIV)-infected patients. Although the risk of morbidity has decreased, it has been replaced by other long-term complications, such as hepatotoxicity. Hepatotoxicity is often reflected in biochemical abnormalities of liver function, such as elevated levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and aspartate aminotransferase-to-platelet ratio index (APRI).

Objective To compare liver function spectrum (AST, ALT, and APRI) in HIV-infected children before and after at least 6 months of HAART.

Methods This observational study (before and after) was conducted in pediatric patients with HIV infection who received HAART for at least 6 months at Sanglah Hospital, Denpasar. Data were collected from medical records.

Results Forty-nine patients were observed in this study. The mean AST, ALT, and APRI levels before HAART were higher than after at least 6 months of HAART. Anti-tuberculosis treatment and fluconazole therapy were not confounding factors for AST, ALT, and APRI.

Conclusion Liver function spectrum enzyme levels of AST, ALT, and APRI are improved after at least 6 months of HAART. **[Paediatr Indones. 2018;58:159-64; doi: http://dx.doi.org/10.14238/pi58.4.2018.159-64]**.

Keywords: *liver function; pediatric; human immunodeficiency virus; antiretroviral*

Human immunodeficiency virus (HIV) infection is a major cause of mortality worldwide. Human immunodeficiency virus-infected patients have increased dramatically during the last decade.[1] Highly active antiretroviral therapy (HAART) has resulted in dramatic decreases in morbidity and improved survival rate in HIV infection.[2] Although the morbidity risk has decreased in the era of HAART, it has been replaced by longer-term, non-traditional morbidity and mortality risks.[3]

The name HAART was introduced in the late 1990s to report the usefulness of combination drug therapies to treat HIV.[4] The HAART recommendations in Indonesia are based on the 2014 Ministry of

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Health decree and are as follows: (1) the first line treatment includes 2 nucleoside reverse transcriptase inhibitors (NRTI) + 1 non-nucleoside reverse transcriptase inhibitor (NNRTI), (2) the first line for children undergoing tuberculosis (TB) treatment are a combination of zidovudine (AZT) or stavudine (d4T) + lamivudine (3TC) + efafirenz (EFV) or other alternatives that are a combination of AZT or d4T + 3TC + abacavir (ABC) or AZT or d4T + 3TC + Nevirapine (NVP), combination AZT/d4T + 3TC + ABC or AZT/d4T + 3TC + EFV or AZT/d4T + 3TC + NVP for children who will start TB treatment, and (3) the first line alternative is tenovofir disoproxil fumarate (TDF) + 3TC/FTC + EFV/NVP.[5]

Though HAART has the potential to slow disease progression, patients may experience several side effects, which include liver toxicity, hematuria, decreased bone density, cardiovascular disease, gastrointestinal tract infection, hypersensitivity reaction, lactic acidosis, and Stevens-Johnson syndrome. Of these complications, the most common is hepatotoxicity.[4] Hepatotoxicity induced by HAART is mostly due to NNRTI, but it can caused by NRTI and protease inhibitor (PI).[5] Mechanisms of drug-induced hepatotoxicity include direct toxicity, hypersensitivity reactions, mitochondrial toxicity, and metabolic abnormalities.[6]

Hepatotoxicity in children with HIV infection is often reflected in biochemical abnormalities of liver function. Biochemical markers commonly used are aspartate aminotransferase (AST) and alanine aminotransferase (ALT) involved in breakdown of amino acids, higher levels of which reflect liver cell injury.[7] Hepatotoxicity can be graded according to the toxicity tables of the AIDS Division of Adults and Pediatrics Adverse Event, and is considered to be present when ALT and AST levels rise above the upper limits of normality (ULN).[8] The scoring system is based on ALT and AST increased as follows: grade 1 (mild) 1.25 to <2.5 x ULN; grade 2 (moderate) 2.5 to <5.0 xfrom ULN; grade 3 (severe) 5.0 to <10.0 x ULN; and grade 4 (potentially life-threatening) ≥10.0 x ULN.[9]

Liver function spectrum studies in children with HIV infection who received HAART have yielded conflicting results. The use of HAART was shown to induce hepatotoxicity after 3 months and even more significantly after 6 months.[6] The HAART may cause liver damage (hepatotoxicity) or have a protective effect on the liver, so it is important to monitor liver function in patients taking HAART. This study was conducted to compare liver function in children with HIV infection before and after receiving HAART for at least 6 months.

Methods

This observational study (before and after) was done to determine liver function (ALT, AST, and APRI score) in children with HIV infection before and after receiving HAART for at least 6 months. Data were collected from medical records in Sanglah Hospital, Denpasar. The target population of this study was children with HIV infection who received HAART for at least 6 months at Sanglah Hospital, Denpasar, Bali. We recruited subjects by consecutive sampling during the year 2012. Patients with incomplete medical records were excluded. This study was approved by the Ethics Committee of Universitas Udayana Medical School.

Collected data were subject characteristics, as well as ALT, AST, and APRI score before HAART and at least 6 months after HAART. Demographic data included address, age, sex, body weight, body height, nutritional status, clinical stage of HIV, and confounding factors which would be controlled by analysis. The confounding factors were hepatitis infection, tuberculosis treatment, and fluconazole therapy.

Subjects aged 18 months to 18 years. Diagnosis of HIV was based on clinical and physical examination, as well as serology markers. The World Health Organization (WHO) classified clinical stage of HIV into stages 1 (asymptomatic), 2 (mild), 3 (moderate), and 4 (severe). The first line antiretroviral therapy for HIV-infected children consisted of NRTI, 3TC, and NNRTI. Liver enzyme levels were assessed by ALT and AST blood tests. Aspartate aminotransferase-to-platelet ratio index (APRI) was calculated by the formula (AST/ULN/platelet count [10^9]) x 100. An APRI threshold of 0.5 was considered to indicate significant fibrosis. Hepatotoxicity was defined as AST and ALT ≥2.5 times above the ULN after 6 months of HAART.

Results

A total of 49 HIV-infected pediatric patients received HAART for at least 6 months at Sanglah Hospital, Denpasar, comprised 29 boys and 20 girls. Most study participants aged 1-5 years. Thirty-six subjects were malnourished and 13 were well nourished. The majority of subjects were in the clinical HIV stage 3-4 group. Subjects' characteristics are shown in **Table 1**.

The mean AST, ALT, and APRI levels before HAART were higher than after at least 6 months of HAART. The mean AST concentration before HAART was 3.4 times higher than the ULN, while that after HAART was only 1.4 times higher than the upper limit normal (ULN), a statistically significant difference (P<0.0001) (**Table 2**). The mean ALT level before HAART was increased 2.03 times from the ULN, but after HAART was within normal limits, however, the difference was not statistically significant (P=0.08) (**Table 3**). The mean APRI before HAART was more than 0.5, but after HAART was within normal limits and significantly different (P<0.0001) (**Table 4**).

Logistic regression was done to analyze for confounding factors of AST, ALT, and APRI levels. The possible confounding factors were hepatitis infection, tuberculosis treatment, and fluconazole therapy. However, there were no subjects with

Table 1. Subject characteristics

Characteristics	(N=49)
Sex, n (%)	
Male	29 (59.2)
Female	20 (40.8)
Age, n (%)	
1-5 years	38 (77.6)
6-18 years	11 (22.4)
Nutritional status, n (%)	
Severe malnutrition	12 (24.5)
Moderate malnutrition	24 (49.0)
Well-nourished	13 (26.5)
Clinical stage of HIV, n (%)	
1-2	17 (34.7)
3-4	32 (65.3)
Hepatitis infection, n (%)	
Yes	0 (0)
No	49 (100)
Tuberculosis treatment, n (%)	
Yes	14 (28.6)
No	35 (71.4)
Fluconazole therapy	
Yes	15 (30.6)
No	34 (69.4)

Table 2. AST level before and after HAART

Variables	Mean (SD)	Mean difference	P value
AST, U/L			
Before HAART	120.28 (171.23)	71.37	<0.0001
After HAART	48.91 (46.14)		

*analysis by Wilcoxon signed-rank test, SD=standard deviation

Table 3. ALT level before and after HAART

Variables	Mean (SD)	Mean difference	P value
ALT, U/L			
Before HAART	70.89 (85.25)	38.77	0.08
After HAART	32.13(29.58)		

*analysis by Wilcoxon signed-rank test, SD=standard deviation

Table 4. APRI before and after HAART

Variables	Mean (SD)	Mean difference	P value
APRI			
Before HAART	3.23 (15.14)	2.90	<0.0001
After HAART	0.33 (0.19)		

*analysis by Wilcoxon signed-rank test, SD=standard deviation

Table 5. Factors associated with elevated liver enzymes were controlled by analysis

Dependent variables	Independent variables	B	95%CI	P value
AST	TB treatment	-1.172	0.034 to 2.793	0.296
	Fluconazole	0.355	0.287 to 7.081	0.664
ALT	TB treatment	0.405	0.098 to 23.069	0.771
	Fluconazole	19.832	0.000 to 0.998	0.998
APRI	TB treatment	1.500	0.833 to 24.086	0.081
	Fluconazole	0.730	0.372 to 11.567	0.405

*Logistic regression test; B=beta coefficient; 95%CI = 95% confidence interval

hepatitis infection. Anti-tuberculosis treatment and fluconazole therapy had no significant correlation to AST, ALT, and APRI, as shown in **Table 5**.

Discussion

In children with HIV infection, HAART may lead to significant hepatotoxicity. Approximately 6 to 30% of treated HIV patients had significantly increased serum liver enzyme levels, which may require discontinuation of treatment.[8] Hepatotoxicity due to HAART may be related to agents from a number of classes, including NRTI, NNRTIs, and PI. The severity of hepatotoxicity may range from transient elevations in transaminase levels to hepatic failure and death, via a variety of mechanisms.[10]

We found that children with HIV infection had liver enzyme elevations up to 3.5 times from the ULN at the time of diagnosis. Both AST and ALT clinically decreased after 6 months of HAART, but the difference was not statistically significant for ALT. This result was in contrast to past studies that suggested the HAART can cause hepatotoxicity.10 Our finding of decreased liver enzymes was consistent with a study in HIV-infected patients in Uganda, which showed few subjects with clinically significant AST elevation during the first three years of HAART. Other studies reported an overall fall in liver enzymes in patients in rural Uganda.[12] Pryce *et al.* also noted that elevations of bilirubin, AST, and ALT prior to commencing HAART could be due to the severity of HIV disease, and these elevations did not necessarily lead to hepatotoxicity after the initiation of HAART.[13] Since HIV is a hepatotrophic virus, the improvement of liver function after HAART initiation could have been due to reduced viral loads, which we did not measure in our study.

Some factors must be considered when comparing our study results to the theory of hepatotoxicity in HAART. Risk factors associated with elevated ALT are high HIV RNA, prolonged HAART exposure, high body mass index (BMI), and increasing age.[14] Aspartate aminotransferase can be transiently elevated, especially in the first 12 weeks of HAART.[11] Most subjects in our study had moderate malnutrition and we evaluated liver enzymes after 6 months of therapy, therefore, overall liver enzyme may have declined by that point.

The lack of APRI elevation risk associated with HAART duration or specific HAART regimen.[15] Nevirapine-based HAART showed AST elevation in few subjects. Other clinical studies reported that NVP was effective and have a good tolerability, but caused an occurrence of characteristic liver injury.[3,11] However, Zidovudine can cause severe hepatotoxicity, and 3TC can lead to hepatotoxic conditions, if they were for long term, especially in patients with chronic hepatitis B.[3] Our subjects used AZT, 3TC, and NVP, and none were infected with hepatitis B.

Human immunodeficiency virus-infected patients are up to 30 times more likely to develop active TB and have a higher risk of dying from TB, than those who are not infected with HIV.[16] Antituberculosis drugs such as rifampicin (RMP), isoniazid (INH), pyrazinamide (PZA), ethambutol (EMB), and streptomycin have good efficacy, but also many adverse effects. Antituberculosis drugs alone can induce hepatotoxicity, which is one of the adverse effects that can cause increased transaminase, bilirubin, icterus, anorexia, nausea, and vomiting.[17] The effect of HAART on TB treatment is not clear.[16] Our study showed that anti-tuberculosis therapy in HIV-infected children did not aggravate hepatotoxicity. A study conducted in both hospitalized patients and outpatients in Jimma, Ethiopia, found that TB treatment induced hepatotoxicity accounted

for a considerable number of cases in TB/HIV co-infected patients.[18] Several studies have shown that anti-TB drugs induced hepatotoxicity between 2-8 weeks.[18,19] We evaluated liver enzymes after 6 months of HAART, so it is possible that we did not detect TB treatment-induced hepatotoxicity in our subjects, because of the time frame.

Malnutrition and low CD4+ level are factors associated with increased risk of developing anti-TB drug-induced hepatotoxicity in HIV-infected children. Malnutrition may be related to depletion of glutathione stores, which makes patients more vulnerable to oxidative injury and slows the pace of liver drug metabolism. The risk of liver toxicity was higher in patients with low baseline CD4+ cell counts, especially those with 50-100 cells/mm^3, than in patients with high CD4+ cell counts. But patients with baseline CD4+ cell counts of <50 cells/mm^3 were not found to have risk of liver toxicity. The absence of an association between CD4+ cell count <50 cells/mm^3 and liver toxicity may be related to the fact that one of the mechanisms involved in the development of hepatotoxicity, the hypersensitivity reaction, which is immunologically mediated, may be less frequently observed in heavily immunosuppressed patients. However, this finding needs to be confirmed and further investigated.[18,19]

Fungal infections are a common cause of morbidity and mortality in HIV-infected patients.20 Fluconazole is commonly used for prophylaxis and treatment of cryptococcosis.[21] Also, HIV-infected patients are particularly at risk of developing local or systemic *Candida albicans* infections. Fluconazole is well established as a first-line management option for both localized and systemic C. *albicans*.[22] Fluconazole is a potent inhibitor of cytochrome P450 isoenzymes and increases the plasma concentrations of a number of co-administered drugs, potentially causing toxicity. One of the first-line drugs for HIV-infected children is NVP. Nevirapine undergoes extensive hepatic metabolism into inactive compounds via P450 isoenzymes, CYP3A4 and CYP2B6. Nevirapine has been associated with severe hepatotoxic reactions and, in some cases, hepatic failure and death.[5,20] Fluconazole significantly raises plasma NVP levels and may cause serious hepatotoxicity.[21]

Our study showed that HAART (containing NVP) and fluconazole, did not increase risk of hepatotoxicity. Wakeham *et al.*[20] and Manosuthi *et al.*[21] showed similar results, as the lack of hepatotoxicity may have been due to serum NVP concentrations of less than 6,000-8,000 ng/mL.[20] We did not measure NVP concentration in our study.

Limitations of this study were that we collected data from medical records and only had single evaluations of liver enzymes. Future studies should be prospective in design, and compared to a gold standard technique such as liver biopsy. More frequent and long-term evaluations, as well as larger sample size, are also recommended for future study.

In conclusion, liver function spectrum (AST, ALT, and APRI) results are improved after at least 6 months of HAART. Antiretroviral therapy effects on TB treatment-induced hepatotoxicity could not be conclusively determined after 6 months of HAART, since anti-TB drugs induce hepatotoxicity between 2-8 weeks. Antiretroviral therapy (containing NVP) and fluconazole do not increase the risk of hepatotoxicity.

Conflict of Interest

None declared.

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Original Article

Valproate use and thyroid dysfunction in children with idiopathic epilepsy

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Abstract

Background Long-term administration of valproic acid (VPA) has side effects, including thyroid dysfunction. Subclinical hypothyroidism (SCH) identified by elevated serum thyroid stimulating hormone (TSH) concentrations with normal thyroxine (T4) and triiodothyronine (T3), or normal free thyroxine (fT4) and free triiodothyronine (fT3) has been demonstrated in idiopathic epilepsy patients receiving VPA.

Objective To evaluate for associations between age at initiation of VPA treatment and duration of treatment with thyroid dysfunction.

Methods A cross-sectional study was conducted from October 2012 to May 2013 in Haji Adam Malik and Pirngadi Hospitals, Medan, North Sumatera. Subjects were children ranging from 0 and below 18 years who had been diagnosed with idiopathic epilepsy. Blood specimens were taken to evaluate serum T3, T4, and TSH levels in all subjects. Data were analyzed using bivariate and multivariate analyses.

Results A total of 49 subjects were included in the study. Age of ≤ 4 years at initiation of VPA was found to be a significant risk factor for SCH in the bivariate analysis (OR 6.67; 95%CI 1.215 to 36.594, P=0.036). Three factors had P values <0.25 in the bivariate analysis and were subsequently analyzed by stepwise multivariate regression test: age at initiation of VPA, duration of treatment, and drug dosage. The VPA initiation at age <4 years had 6.67 times the risk of SCH than the age of >4 years (95%CI 1.215 to 36.594; P=0.029). Duration of treatment and VPA dosage were not significantly associated with SCH on multivariate analysis

Conclusion Age ≤ 4 years old at the initiation of VPA is associated with thyroid dysfunction. However, no significant association was found between duration of treatment as well as drug dosage with thyroid dysfunction. **[Paediatr Indones. 2018;58:192-7; doi: http://dx.doi.org/10.14238/pi58.4.2018.192-7]**.

Keywords: *idiopathic epilepsy; thyroid dysfunction; valproic acid; subclinical hypothyroidism*

Epilepsy is a chronic condition characterized by recurrent seizures and occurring with or without stimulation. Epilepsy is common in children, with an incidence of 50 new cases per 100,000 population.[1] Idiopathic epilepsy is genetically determined and has no apparent structural cause.[2] The main treatments for epilepsy are antiepileptic drugs (AEDs), chosen in accordance with the frequency of seizures.[3] Thyroid hormone homeostasis can be impaired by phenytoin, phenobarbital, and carbamazepine, as potent inducers of cytochrome P450 enzymes.[4] In contrast, the mechanism of thyroid dysfunction by valproic acid (VPA) remains unclear.[5,6]

Subclinical hypothyroidism (SCH) is a common side effect of VPA use. A study in India reported that the prevalence of subclinical hypothyroidism in idiopathic epilepsy with VPA monotherapy was 26%.[7] Subclinical hypothyroidism is marked by

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an increase in TSH accompanied by normal free thyroxin (ft4) and free triiodothyronine (ft3), or thyroxine (t4) and triiodothyronine (t3). These circumtances are not always followed by significant clinical manifestations, but should not be ignored, as impaired neurodevelopment leads to defects in cogitive function, particularly in children.[8] Several possible risk factors for thyroid function disorders are the age at initiation of VPA, duration of VPA use, and concomitant use of VPA with other AEDs, like carbamazepine.[9] The aim of our study was to evaluate for possible associations between thyroid dysfunction with age at initiation of VPA and duration of treatment.

Methods

A cross-sectional study was conducted in Haji Adam Malik and Pirngadi Hospitals from October 2012 to May 2013. Inclusion criteria were children diagnosed with idiopathic epilepsy, aged below 18 years, and who received VPA as antiepileptic monotherapy. We excluded children diagnosed with endocrine, heart, renal, or liver disease, those who used drugs known to interfere with thyroid function, and those with family history of hypothyroidism or hyperthyroidism.

History of some clinical manifestation of subclinical hypothyroidism such as short stature, alteration of the school yield, skin dries, fragile hair, and muscle pain was taken from parents and subjects. Body weight (BW) was measured by *Camry®* scales and body height by microtoise. Blood specimens (3 mL) were obtained from the median cubital vein, without anticoagulant, using a 3 mL syringe. Serum thyroid was measured using an automatic *COBAS e601 Roche Diagnostic®* (Swiss) with electrochemistry immunoassay (ECL). Serum thyroid concentration was reevaluated 2 weeks after the first examination in patients suspected to have thyroid dysfunction. Thyroid hormone levels were compared to the reference values from the *Unit Kerja Koordinasi (UKK) Endokrinologi IDAI (Endocrinology Working Group of Indonesian Pediatric Society)*.[10]

Data were processed and analyzed using *SPSS version 15.0* software. Statistical analyses used were Chi-square, Fisher's exact, and Mann-Whitney tests, with significance at $P < 0.05$. All risk variables with $P < 0.25$ in the bivariate analysis were included in the multivariate analysis. Variables were assessed with binary logistic regression with significance value of $P < 0.05$ and 95%CI. Subjects' parents provided written informed consent. This study was approved by the Ethics Committee of the Universitas Sumatera Utara Medical School.

Results

Of 53 children who visited the outpatient clinics, 4 were excluded. Two of these children had consumed drugs that can interfere with thyroid function (ferrous sulphate and mefenamic acid), and the other 2 children had severe disease (dengue shock syndrome and brain injury). Hence, 49 subjects underwent serum thyroid examinations.

Table 1. Subjects' characteristics

Characteristics	(N = 49)
Sex, n (%)	
Male	27 (55.10)
Female	22 (44.90)
Mean height (SD), cm	122.5 (23.80)
Mean weight (SD), kg	28.3(15.41)
Type of epilepsy, n (%)	
Idiopathic general epilepsy	44 (89.80)
Idiopathic partial epilepsy	5 (10.20)
Age at initiation of VPA, n (%)	
≤ 4 years	11(22.40)
> 4 years	38(77.60)
Duration of treatment, n (%)	
< 6 months	5 (10.20)
6-24 months	29 (55.20)
>24 months	15 (30.60)
Mean drug dosage (SD), mg/kg BW	22.3 (3.59)

Mean age at VPA initiation was 7.82 years. The majority of children were >4 years old (77.6%). Most subjects had general idiopathic epilepsy (89.8%). Mean daily dosage of VPA was 22.3 (SD 3.59) mg/kg BW. Most subjects had a 6-to-24-month duration of treatment (55.2%). None of the subjects had below normal height, according to the *Centers for Disease Control and Prevention* (CDC) growth chart for children > 5 years,[11] and the Z-score chart for children ≤ 5 years.[12]

Subclinical hypothyroidism was found in 7/49 (14.3%) subjects (TSH level higher than normal and T3 and T4 within normal limits). In the subclinical hypothyroidism group, mean TSH levels were 6.77 (SD 1.54) μIU/mL in the prepubertal group (age 1 to 10 years old), and 9.29 μIU/mL in the pubertal group (10 to below 18 years, higher than the euthyroid group (mean TSH levels were 2.71 (SD 1.15 in prepubertal group and 2.61 (SD 0.99) in pubertal group. None of the subjects had serum T3 and T4 under and above normal limits.

We evaluated several potential risk factors for subclinical hypothyroidism. Age <4 years at VPA initiation was the only significant risk factor for SCH (P=0.036). Duration of treatment, type of epilepsy, daily VPA dosage, and sex were not significantly associated with SCH (**Table 2**).

Three factors had P values <0.25 in the bivariate analysis: age at VPA initiation, duration of treatment, and VPA dosage. Stepwise multivariate analysis with logistic regression model revealed that VPA initiation at age <4 years had 6.67 times the risk of SCH than the age of >4 years (95%CI 1.215 to 36.594; P=0.029). Duration of treatment and VPA dosage were not significantly associated with SCH on multivariate analysis (**Table 3**).

None of our subjects complained of any clinical manifestation associated with subclinical hypothyroidism, such as short stature, lack of the concentration, dry skin, fragile hair, or muscle pain.

Discussion

In our study, the mean age at VPA initiation was 7 years, with subjects ranging less than 18 years old. Characteristics of our subjects were similar to a 2006 Spanish study of 23 patients who underwent VPA treatment, but they did not specify epilepsy type or neonatal convulsion. It has found an increase of the serum levels of TSH in 7 of 23 patients (30.43%) between 5.85 and 9.24 mUI/L.[13] Our study involved 49 subjects, consisted of only idiopathic epilepsy, and received VPA as AED monotherapy.

Subclinical hypothyroidism was observed in 7 subjects (14.3%). A Lebanese study reported that

Table 2. Bivariate analysis of possible risk factors and subclinical hypothyroidism

Variables	Subclinical hypothyroidism (n=7)	Euthyroid (n=42)	OR	95% CI	P value
Age VPA initiation, n (%)					
≤ 4 years	4 (57.1)	7 (16.7)	4.61	1.22 to 36.6	0.036
> 4 years	3 (42.9)	35 (83.3)	-	-	
Duration of treatment, n (%)					
< 6 months	0	5 (11.9)	1.32	1.07 to 1.62	0.06
6-24 months	7 (100)	22 (52.4)	-	Ref	
> 24 months	0	15 (35.7)	0.76	0.62 to 0.93	
Type of epilepsy, n (%)					
Idiopathic general epilepsy	7(100)	37 (88.1)	0.85	0.75 to 0.97	1.000
Idiopathic partial epilepsy	0	5 (11.9)	-	-	
Mean VPA dosage (SD), mg/kg	24.1 (2.41)	22.0 (3.69)	-	-	0.087
Sex, n (%)					
Male	5 (71.4)	22 (52.4)	2.04	0.44 to 9.50	0.436
Female	2 (28.6)	20 (47.6)	-	-	

Table 3. Multivariate analysis of possible risk factors and subclinical hypothyroidism

Risk factors	Coefficient	Adjusted OR	95% CI	P value
Age at the first VPA use	1.897	6.67	1.215 to 36.594	0.029
Duration of treatment	-1.020	0.304	0.052 to 2.518	0.304
Valproic acid dosage	0.165	1.180	0.900 to 1.546	0.231

25.2% of patients using VPA as an antiepileptic experienced subclinical hypothyroidism compared to control.[14] Another study in India showed a SCH prevalence of 26%.7 In contrast, two studies reported no impaired thyroid function after the use of antiepileptics. An Iranian study concluded that serum thyroid levels were within normal limits after use of phenobarbital, carbamazepine, valproic acid, and primidone for 2 months.[15] Also, an Italian study concluded that VPA monotherapy did not have a significant effect on thyroid disorders.[16]

The lower percentage of patients with subclinical hypothyroidism in our study compared to that of past studies may have been due to different definitions of subclinical hypothyroidism. There are study used VPA only, and some study use some of AED. We used reference values from the *Endocrinology Working Group of Indonesian Pediatric Society*, which differentiates normal serum thyroid based on age. The definition of normal TSH has long been debated and TSH level seems to vary widely in different populations and according to different study methods.[17]

The underlying mechanism of VPA effect on thyroid levels may be due to the γ-aminobutyric acid-stimulating properties of VPA. The γ-aminobutyric acid inhibits the release of somatostatin, which inhibits TSH secretion. A secondary factor is exhibited by zinc and selenium deficiencies result on malfunction of the human 5'deiodinase.[9,14] A longitudinal, controlled study revealed alterations in serum thyroid profiles were associated with low serum copper.[18] We did not evaluate for mineral deficiencies in our subjects, thus, we cannot comment on these potential effects.

Several factors reportedly play a role in causing thyroid dysfunction in patients with epilepsy who use VPA as an antiepileptic drug. An Indian study reported such factors to be the age of the child at VPA initiation, long-term use, and antiepileptic polytherapy use.[7]

We found that age <4 years at VPA initiation was a significant risk factor for SCH, with a 6.67 times increase than in children >4 years old (multivariate analysis: 95%CI 1.215 to 36.594; P=0.029). Previous studies also found that younger age was associated with subclinical hypothyroidism.[9,13,14] Duration of treatment was not a significant factor in our study, as most subjects were in the 6-24-month duration of treatment, and all subjects with SCH were in this group. In contrast, Kim *et al.* reported a higher prevalence of thyroid disorders than in our study.[9] In addition, an Italian study in 2006 found that serum T4, fT4, T3, fT3 and rT3 levels decreased in the third and sixth month while on oxcarbazepine, whereas those using VPA had serum T4, fT4, T3, FT3, and RT3 levels almost equal to the base value, but increased mean serum TSH level, especially after 6 months of use. Those who used oxcarbazepine did not experience a significant change in serum TSH. This finding suggests that thyroid dysfunction may occur after 6 months of VPA use.[19]

A Korean study also assessed several other factors such as serum levels of VPA, phenobarbital and carbamazepine, gender, type of epilepsy, and drug dosage. Among these factors, only serum VPA level was associated with thyroid malfunction.[9] We also conducted an analysis of gender, epilepsy type, and drug dosage, but found no significant associations between these factors and SCH. Measurement of serum VPA levels was not performed on our study due to unavaibillity of facilities.

Subclinical hypothyroidism can lead to clinical symptoms, such as growth failure and cognitive impairment, when the patient falls into a state of overt hypothyroidism. Monitoring of thyroid hormone levels is imperative so that therapy can be administered immediately, especially in high-risk groups, namely under the age of two years.[8] In our study, subjects had no clinical manifestations of hypothyroidism.

Several studies have shown that treatment of subclinical hypothyroidism can reduce the risk of overt hypothyroidism.[20,21] Currently, there is no consensus on management of subclinical hypothyroidism in patients using VPA. Regular monitoring of serum thyroid levels every six months should be performed.[20] For the treatment of subclinical hypothyroidism in patients with manifestations, administration of L-thyroxine can be considered, as it may prevent atherosclerosis and intellectual impairment.[21]

To our knowledge, this study is the first conducted in Indonesia to assess the risk factors for thyroid dysfunction in patients with idiopathic epilepsy using VPA monotherapy as an AED. Moreover, this study focused on the use of VPA as the antiepileptic drug. Our results show that despite relative safety and stability, the use of VPA monotherapy at the age of <four years may increase the risk of thyroid

dysfunction in patients with idiopathic epilepsy.

A limitation in our study was that the baseline TSH level was not measured before VPA treatment started. Also, a more complete examination such as evaluating serum antithyroid antibodies, zinc, copper, and selenium was also not performed due to lack of measurement tools and facilities in Haji Adam Malik Hospital. Another limitation was the broad age range of the subjects, because thyroid dysfunction may have more significant effects on the development of children at younger ages. Indeed, the risk factor for the occurrence of thyroid dysfunction was VPA initiation at <4 years of age. Although there were no clinical manifestations that arose due to subclinical hypothyroidism, VPA use at a younger age should be accompanied by regular monitoring in order to avoid overt hypothyroidism which can cause developmental disorders in children.

In conclusion, age <4 years at valproic acid initiation is significantly associated with thyroid dysfunction. However, no association was found between duration of treatment as well as drug dosage with thyroid dysfunction. Further studies should be done with a larger sample size scale and prospective cohort design.

Conflict of Interest

None declared.

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Original Article

Lung function test in children with left-to-right shunt congenital heart disease

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Abstract

Background Increased pulmonary blood flow may lead to abnormal lung function in children with left-to-right (L to R) shunt congenital heart disease. This condition has been linked to considerable mortality and morbidity, including reduced lung function.

Objective To assess for lung function abnormality in children with L to R shunt congenital heart disease.

Methods We conducted a cross-sectional study involving children aged 5-18 years and diagnosed with L to R shunt congenital heart disease at Dr. Sardjito Hospital from March to May 2017. Subjects underwent spirometry tests to measure forced expiratory volume-1 (FEV-1), forced vital capacity (FVC), and forced expiratory volume-1 (FEV-1)/forced vital capacity (FVC).

Results Of 61 eligible subjects, 30 (49.2%) children had atrial septal defect (ASD), 25 (41%) children had ventricular septal defect (VSD), and 6 (9.8%) children had patent ductus arteriosus (PDA). Spirometry revealed lung function abnormalities in 37 (60.7%) children. Restrictive lung function was documented in 21/37 children, obstructive lung function in 11/37 children, and mixed pattern of lung function abnormality in 5/37 children. Pulmonary hypertension was found in 21 children. There was no significant difference in lung function among children with and without pulmonary hypertension (P=0.072).

Conclusion Abnormal lung function is prevalent in 60.7% of children with L to R shunt congenital heart disease, of which restrictive lung function is the most common. There was no significant difference in lung function among children with and without pulmonary hypertension. **[Paediatr Indones. 2018;58:165-9; doi: http://dx.doi.org/10.14238/pi58.4.2018.165-9]**.

Keywords: *congenital heart disease; L to R shunt; spirometry; lung function*

Congenital heart disease (CHD) is a significant health problem, with the highest birth prevalences seen in low- and middle-income coountries.[1,2] Left-to-right shunt CHD results in increased pulmonary blood flow (Qp) beyond the regular systemic blood flow (Qs).[3] The pathophysiological changes depend on the size of the defect and volume of pulmonary blood flow (Qp), which have been associated with increasing pulmonary extracellular fluid. Other factors affecting the magnitude of shunts include defect location, patient age, and pressure gradient between the two chambers of the shunt.[4,5] Lung function abnormality in children with CHD are due to either structural impact on the airways, or abnormal pathophysiological mechanisms leading to increased lung fluid and/or significant pulmonary disease.[6]

Children with CHD are at greater risk of infection including those of the respiratory tract.[7] Respiratory problems are linked to considerable morbidity and

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mortality in children.[6] A previous study reported that moderate to severe impairment of lung function was an independent predictor of mortality among adults with CHD [hazard ratio (HR) 1.63; 95% confidence interval (CI) 1.01 to 2.63; P=0.004].[8] However, there has been limited study on lung function abnormality in children with (CHD).[9] We aimed to assess lung function abnormalilty in children with L to R shunt congenital heart disease.

Methods

This cross-sectional study involved children aged 5 to <18 years and diagnosed with L to R shunt congenital heart disease at Dr. Sardjito Hospital from March 1 to May 31 2017. Congenital heart disease was confirmed using echocardiography. We included all children who had never undergone definitive treatment of the defect. Subjects underwent spirometry testing. Children with acute and chronic disorders affecting spirometry performance, such as vomiting, vertigo, hemoptysis, chronic lung disease, recent eyes, or abdominal or thorax surgery were excluded. We obtained informed consent from all subjects' parents.

Demographic data, history of medical treatment, and echocardiography results were collected from medical records. Spirometry was performed using a vitalograph spirometer pneumotrac type 6800 to assess FEV1, FVC, and FEV-1/FVC. Spirograms were accepted if they were free from artifacts, had good starting effort and showed satisfactory exhalation.[10] Lung function abnormalities were classified following the plot (**Figure 1**).

Data were analyzed using *SPSS version 22.0* for Windows 2007, and presented as mean, median, or proportion, as appropriate. Chi-square test was used to analyze the results when comparing proportions. A probability value of less than 0.05 was considered to denote statistical significance. This study was approved by the Medical and Health Research Ethics Committee of Universitas Gadjah Medical School/Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

Results

Of 61 eligible subjects, 30 (49.2%) children had ASD, 25 (41%) children had VSD, and 6 (9.8%) children had PDA (**Table 1**). Lung function abnormality was observed in 37 (60.7%) patients. Restrictive lung function was found in 21 (56.8% of those with lung function abnormality) patients, consisting of 14/21 mildly restrictive, 4/21 moderately restrictive, and 3/21 (14.3%) severely restrictive lung function abnormalities.

Eleven (29.7%) patients had obstructive lung function, out of which 90.9% had mild obstructive lung fuction and 9.1% had moderate obstructive lung function. Mixed type lung function abnormality was found in 5/37 patients.

Seventeen of 30 (56.7%) children with ASD had lung function abnormality. In the VSD group, lung function abnormality was found in 15/25 (60%) children. Of children with PDA, 5/6 (83%) had abnormal lung function, mostly of the restrictive type. **Figure 2** describes the prevalence of lung function abnormalities for each type of congenital heart disease.

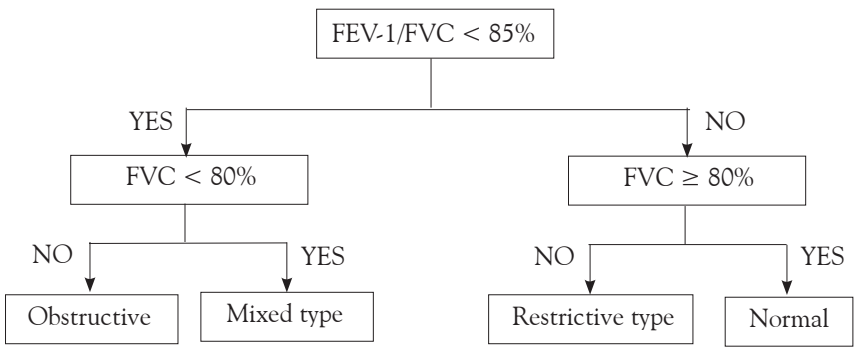

Figure 1. Interpretation of spirometry results[11]

Table 1. Baseline characteristics of subjects

Characteristics	(N=61)
Male sex, n (%)	28 (46)
Median age (range), years	10 (5-18)
Congenital heart disease, n (%)	
Atrial septal defect	30 (49)
Ventricle septal defect	25 (41)
Patent ductus arteriosus	6 (10)
Pulmonary hypertension, n (%)	21 (34)
Nutritional status, n (%)	
Good	37 (61)
Wasted	12 (20)
Severely wasted	11 (18)
Overweight	1 (1)

describes the results of the Chi-square analysis of lung function abnormality and pulmonary hypertension in our subjects.

Discussion

Defects at the great artery, atrial, or ventricular level which result in left-to-right shunts permit excess blood flow from the systemic circulation to the pulmonary circulation. This excessive pulmonary blood flow is linked to lung function abnormality in children with CHD.[5,7] Our study showed that 37/61 (60.7%)

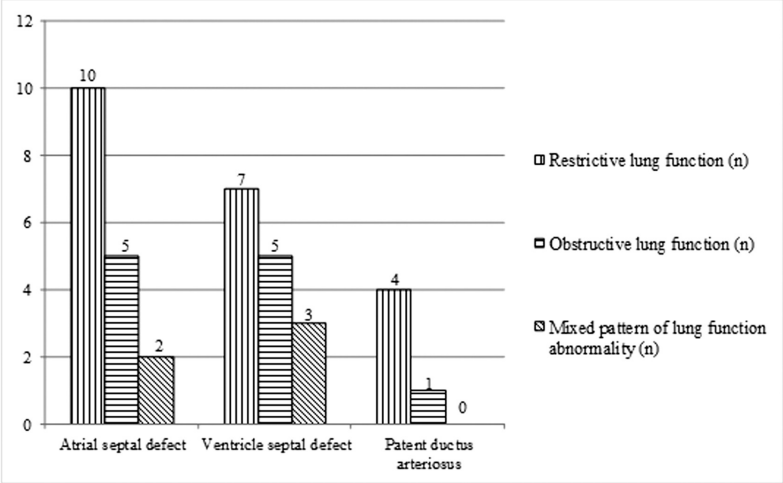

Figure 2. Lung function abnormality in each type of congenital heart disease

Based on echocardiography results, 21/61 left-to-right shunt CHD patients were diagnosed with pulmonary hypertension. The restrictive type was the most common lung function abnormality in subjects with pulmonary hypertension. There was no significant difference in lung function among children with and without pulmonary hypertension (P=0.072). **Table 2**

Table 2. Analysis of lung function abnormality and pulmonary hypertension in children with L to R shunt CHD

Pulmonary hypertension	Lung function, n (%)		P value
	Normal	Abnormal	
Yes	5 (23.8)	16 (76.2)	0.072
No	19 (47.5)	21 (52.5)	

children with CHD had lung function abnormalities. Yau *et al.* found that infants with CHD and left-to-right shunts had lower lung compliance and higher expiratory airway resistance than normal children (P<0.001).[12]

In our study, the most common lung function abnormality was the restrictive type, in 21 (56.8%) of subjects with lung function abnormalities. Similarly, Ginde *et al.* reported that restrictive lung function was prevalent in adults with CHD.13 Patients with a history of CHD tend to have mild to moderate restrictive changes, resulting in smaller lung volumes and flow rates relative to healthy subjects, leading to reduced ventilatory capacity, on average.[14]

Engorged vessels and volume-loaded heart chambers caused by left-to-right shunt CHD

may lead to external bronchial compression and obstructive lung function. Common sites of compression are the left main bronchus below the carina, which are compressed between an enlarged left atrium posteriorly and a dilated pulmonary artery or PDA anteriorly.[4,7] Partially obstructed airways develop a ball-valve effect and cause prolonged expiration. Completely obstructed airways lead to segmental atelectasis. The peak incidence is in infancy, when the bronchial cartilage is soft.[4] Only 11/61 children (18%) had obstructive lung function in this study. Lung function results may differ among age groups.

We found that 17/30 of patients with atrial septal defects had lung function abnormalities. This finding was consistent with a previous study in which 18/26 patients with ASD who had never undergone intracardiac correction had lung function abnormalities.[15]

Sari et al. performed spirometry in 20 children with VSD and found that 15/20 had restrictive lung function.[9] Similarly, 15/25 children with VSD had lung function abnormalities, of which 7/15 were the restrictive type. The larger the defect size, the higher the flow ratio. And more frequent respiratory tract infection in children with VSD increases the risk of restrictive lung function.[9]

Diagnosis of pulmonary hypertension in this study was established by echocardiography results. Echocardiography is used as a non-invasive method for assessing structural and functional intracardiac abnormalities, and monitoring disease progression over time. The sensitivity and specificity of echocardiography for pulmonary hypertension was 83% (95%CI 73 to 90) and 72% (95%CI 53 to 85), respectively.[16] A limitation of our study was that more accurate diagnoses of pulmonary hypertension should be done by right heart catheterization as the gold standard. Another limitation was not analyzing factors that may affect lung function abnormality in children with left-to-right shunt congenital heart disease.

In conclusion, abnormal lung function is prevalent in children with left-to-right shunt congenital heart disease, of which restrictive lung function is the most common. There is no significant difference in lung function among subjects with and without pulmonary hypertension.

Conflict of interest

None declared.

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Original Article

Lung function in athletes and non-athletes aged 13-15 years

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Abstract

Background Regular sports or physical training contributes in increasing the body's pulmonary function. The increase of pulmonary function is determined by the strength of respiratory muscle, thoracic compliance, upper respiratory system resistance, and pulmonary elasticity.

Objective To compare pulmonary function between athletes and non-athletes aged 13-15 years.

Methods This is a cross-sectional analytical study conducted on junior high school students aged 13-15 years throughout June to August 2017. Participants are classified as athletes from particular sports and non-athletes. Assessment of pulmonary function was done using a spirometry test, in which each subject was asked to inhale and exhale in a particular method. Parameters assessed include vital capacity (VC), forced vital capacity (FVC), expiratory volume in 1 second (FEV1), forced expiratory flow (FEF) and FEV1/FVC. Differences in lung function between athletes and non-athletes were analyzed using independent T-test.

Results There were 60 athletes and 60 non-athletes included in this study. The mean age of athletes and non-athletes were 13.38 (SD 0.99) years old and 13.70 (SD 0.76) years old, respectively. The statistically significant differences in mean lung function parameters between athletes and non-athletes were as follows: VC: 85.03% *vs.* 79.41%, respectively (P=0.035); FVC: 95.66% *vs.* 88.43%, respectively (P=0.016); FEV1: 102.10% *vs.* 94.28%, respectively (P=0.016); and FEV1/FVC: 105.95% *vs.* 102.69%, respectively (P=0.011). However, there were no statistically significant differences in the means of FEF 25-75% between the two groups (P>0.05).

Conclusions Parameters of lung function in athletes are in general significantly higher than in non-athletes. **[Paediatr Indones. 2018;58:170-4; doi: http://dx.doi.org/10.14238/pi58.4.2018.170-4]**.

Keywords: *lung function; athletes; non-athletes*

Regular sports or physical training may increase the body's physiologic capacity, including respiratory function.[1] The respiratory system is one of the most important body systems, in which effective gas exchange is required, especially during physical activity. Human lungs are sensitive to conditions that result in increased aerobic metabolism such as running, cycling, and swimming.[1,2] The type, intensity, and duration of regular exercise that athletes practice result in different lung function measures.[3] During intense physical activity, oxygen consumption as measured by maximal aerobic metabolism (VO_2 max) increases. The VO_2 max reflects the total amount of oxygen that can be utilized during physical training (measured in mL O_2/kg body weight/min). Practicing 7 to 13weeks of physical training may increase the VO2 max by more than 10%.[4]

Determinants of pulmonary function include: strength of respiratory muscles, thoracic compliance, upper respiratory system resistance, and pulmonary elasticity.[1,5] Respiratory function increases as

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children grows and develops. Pre-pubertal boys and girls have similar lung physiology, both at rest and during exercise. Significant development of strength, flexibility, and equilibrium occurs at ages 6-12 years. Above this age range, child development will be quite robust including its physical characteristics and movement.[6,7]

Respiratory muscle growth, lung expansion capability, thoracic cavity and bronchial elasticity, and adequate bronchiolus function may increase lung function thoroughly, in terms of vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), ratio of FEV1 and FVC (FEV1/FVC), and forced expiratory flow (FEF). Pulmonary function is examined using spirometry. Spirometry results are interpreted as normal, (normal FVC and FEV/FVC), restrictive (decreased FVC and normal or increased FEV1/FVC), and obstructive (decreased FVC and FEV1/FVC ratio).[5,6] Physical inactivity is a major cause of most chronic conditions, including decreased cardiorespiratory fitness (CRF), in terms of capacity of the cardiovascular and respiratory systems to supply oxygen-rich blood to the working skeletal muscles and the capacity of the muscles to use oxygen to produce energy for movement.[2] Regular physical training may have a positive influence on respiratory and circulatory function, as a benchmark of physical fitness and increased quality of life.[5,8,9] The aim of this study was to compare pulmonary function in athletes and non-athletes aged 13 and 15 years.

Methods

This cross-sectional, analytic, observational study was conducted on junior high school students aged 13-15 years, who were classified as athletes and non-athletes (of particular sports) throughout June to August 2017. Subject selection is conducted using two-stage cluster sampling. We randomly chose two junior high schools from a list obtained from the 'Dinas Pendidikan, Pemuda dan Olahraga' (Education, Youth, and Sport Office) of Denpasar, Bali. A total of 120 subjects from the two schools were included, 60 athletes and 60 non-athletes. Parents consented to their children's participation in this study. Exclusion criteria include obesity, presence upper respiratory tract infection at time of study, history of active and/or passive smoking, history of reduced lung function, such as due to a chronic cough, or recurrent upper respiratory tract infection, abnormal heart function, obstructive lung diseases, such as asthma or thoracic cavity abnormality that may influence the lung volume. Subjects underwent anthropometric and spirometry tests. The lung function test was conducted using a single-blind technique. This study was approved by the Medical Ethics Committee of Udayana University, Sanglah Hospital, Denpasar.

An athlete was defined as someone who had an affinity for particular sports, who competed in those or other sports, and who conducted routine training with a minimum of running 2 kilometers every 2 days per year or swimming at least 300 meters, according to the same schedule. A non-athlete was defined as those without an affinity for sports, did not have competitive experience in a sport, and did not include exercise as a hobby.

Lung function was defined as the ability of the lung to conduct gaseous exchange, in which oxygen was absorbed and carbon dioxide was released, as measured by spirometry. Spirometry is a tool to measure air movement in or out of the lungs during a particular lung maneuver. Vital capacity was defined as the volume of air expired slowly from the end of maximal inspiration until the end of maximal expiration. Forced vital capacity was defined as the speed of expiration flow and the length of inspiration time. The FVC maneuver was done by maximal inspiration, followed by quick and forceful expiration. This maneuver was also used to measure other parameters, such as forced expiratory volume in 1 second (FEV1), FEV1/FVC, and FEF. The FEV1 was defined as the volume of forced expiration in the 1st second. The FEV1/FVC was defined as the ratio between FEV1 and FVC, and the FEF was defined as the speed of air expired during the middle portion of a forced expiration, from 25% to 75%, and considered to be the remaining fraction of the FVC.[10]

Body weight (measured in kg) was measured using a standing scale, with the subject being in a state of minimal clothing, without wearing shoes. Body height (measured in cm) was measured using plastic rulers attached to the wall, with subjects standing upright, back against the wall, without wearing shoes. Body mass index (BMI) was defined as body weight divided by body height squared (kg/m^2).

The data collected included age, sex, height, weight, sports championship history, and length of training. Unpaired mean sample size formula was used to calculate the minimum required sample size, which was 59 subjects in each group. Independent T-test was used for lung function analysis because the distribution of data was normal. Results with P values <0.05 were considered to be statistically significant. Statistical analysis was calculated using *SPSS 18.0 for Windows*.

Results

Out of the 178 adolescents screened for this study, 58 of them were excluded: 23 were screened to have obstructive lung diseases such as asthma, 16 were passive smokers, 12 had upper respiratory tract infection, and 7 were obese. Hence, 60 athletes and 60 non-athletes participated in this study. The mean age was 13.38 (SD 0.99) years for athletes group and 13.70 (SD 0.76) years for non-athletes group. The characteristics of study subjects are shown in **Table 1**.

Table 1. Characteristics of study subjects

Characteristics	Athletes (n = 60)	Non-athletes (n = 60)
Sex, n (%) Male	30 (50)	30 (50)
Mean age (SD), years	13.38 (0.99)	13.70 (0.76)
Mean body weight (SD), kg	48.77 (10.51)	49.67 (12.45)
Mean height (SD), cm	153.80 (7.92)	154.94 (8.71)
Mean BMI (SD), kg/m^2	20.45 (3.03)	20.54 (3.91)
Types of sports, n (%)		
Athletics	18 (30)	
Basketball	21 (35)	
Football	9 (15)	
Swimming	12 (20)	

Table 2 shows the differences in lung function parameters between the two groups. The athlete group had significantly higher mean VC, FVC, FEV1, and FEV1/FVC than the non-athlete group. However, mean FEF 25, FEF 50, and FEF 75 were not significantly different between groups.

Discussion

Lung function may be influenced by genetic, environment, and nutritional factors. Routine physical activity during childhood growth and development may increase the lung-muscle endurance. Routine physical activity during adolescence may increase hyperplasia of alveolar tissue, the formation of new alveoli, and lung microcirculation.[11,12] Athletes undertaking high intensity physical training have increased lung function compared to non-athletes. Several Indian studies showed that the duration of physical activity that influence lung function was between 1 and 8 months.[11,12] Several other studies showed that lung function in males is better than in females, possibly due to differences in thoracic cage size and muscle strength.[13,14]

Routine physical activity may also increase production of contractile protein, including actin and myosin. Increased muscle contraction strength may increase an athlete's lung function compared to that of non-athletes, because of lung changes in muscle strength, expansion, elasticity, and equilibrium.[15] We used spirometry to measure lung function parameters in our subjects. Vital capacity (VC) is measured using a combination of lung dimension, lung compliance, and respiratory muscle strength. We found that athletes had mean VC of 85.03%, which was significantly higher than that of non-athletes (79.41%; P=0.035). A study

Table 2. Comparison of lung function parameters between athletes and non-athletes

Variables	Athletes (n=60)	Non-athletes (n=60)	Mean difference (95%CI)	P value
Mean VC (SD), %	85.03 (13.79)	79.41 (15.10)	5.62 (0.39 to 10.85)	0.035
Mean FVC (SD), %	95.66 (14.03)	88.43 (18.07)	7.23 (1.38 to 13.07)	0.016
Mean FEV1 (SD), %	102.10 (13.92)	94.28 (20.56)	7.80 (1.45 to 14.15)	0.016
Mean FEV1/FVC (SD), %	105.95 (5.86)	102.69 (7.87)	3.26 (0.74 to 5.77)	0.011
Mean FEF25 (SD), %	93.25 (22.6)	92.89 (20.26)	0.35 (-7.41 to 8.13)	0.927
Mean FEF50 (SD),%	111.68 (30.72)	111.64 (24.15)	0.04 (-9.95 to 10.03)	0.994
Mean FEF75 (SD), %	127.31 (43.25)	123.42 (42.6)	3.89 (-11.63 to 19.42)	0.620

in India showed that among adults measurements after exercise, there was a significant increase in VC in both athletes and non-athletes.[10]

The FVC was measured by maximal inspiration followed by maximal expiration.[16] We found that the mean FVC in athletes was significantly better (95.66%) compared to that of non-athletes (88.43%). Similarly, Vedala *et al.*[17] and Mahotra[18] stated that the FVC in athletes was significantly higher than in non-athletes. Muscular exercise increases the rate and depth of respiration and improves FVC, the consumption of O_2, and the rate of diffusion. Physical activity was observed to be positively correlated to the changes of FVC between ages 13-27 years.[7]

The FEV1/FVC ratio is used as a marker for obstructive and restrictive conditions of the lungs.[18] The mean FEV1/FVC in athletes (105.95%) was significantly higher compared to that of non-athletes (102.69%; P=0.011), similar to results from Vedala *et al.*[17] However, Akhade *et al.* found no significant difference in mean FEV1/FVC percentage between athletes and non-athletes.[19] Regarding the FEF of 25-75%, we found no statistically significant differences between athletes and non-athletes.

General lung function test revealed an overview of lung function in athletes compared to non-athletes, with significantly higher VC, FVC, FEV1, and FEV1/FVC values in the athlete group. A Turkish study conducted in 15-16-year-olds also noted significant differences of lung function between regular exercise and sedentary individual or inactive individual.[20] Regular exercise training is thought to increase the oxygen demand in working muscles, which stimulate the respiratory centers in the brain stem and send strong signals to the inspiratory muscle group. These muscles cause forceful inspiration and expiration, as well as increased secretion of surfactant and prostaglandin (PGE2) in the alveolar space to decrease alveolar surface tension and decrease physiological dead space. Consequently, these actions are reflected as increased pulmonary function in atheletes. Increased lung compliance and decreased bronchial smooth muscle tone tend to increase the general lung function of athletes.[15,19] Our study may be indicative of changes in lung and muscle function, thoracic cage movement, and equilibrium between lung and thoracic cage elasticity in response to regular physical activity. Limitation of this study was that we only performed lung function test one time and not evaluating physical activities before spirometry test that might influence the test.

As a conclusion, lung function in athletes is significantly higher compared to non-athletes, in terms of VC, FVC, FEV1, and FEV1/FVC. However, FEF 25-75% are not significantly different between athletes and non-athletes. Routine physical activity may have positive effects on lung capacity. More studies should be done on the differences between lung function in athletes from various sports.

Conflict of Interest

None declared.

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Original Article

Hemostatic abnormalities in children with thalassemia major and liver iron overload

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Abstract

Background Thalassemia major (TM) patients are susceptible to liver dysfunction due to iron deposition. Pediatric TM patients often present with bleeding. Blood loss necessitates transfusions, leading to increased iron absorption from the gut.

Objective To study hemostatic abnormalities in children with TM and iron deposition in the liver.

Methods This cross-sectional study involved 190 non-splenectomized children with TM. Liver iron deposition was evaluated using T2* MRI. Prothrombin time (PT), activated partial thromboplastin time (aPTT), and platelet counts were assessed from blood specimens.

Results Most subjects were diagnosed with β-thalassemia and β-thalassemia/HbE. The majority of subjects were on deferiprone (DFP) treatment. Approximately 89.5% of subjects had liver iron overload. Prolongation of PT and aPTT, as well as thrombocytopenia were observed in 60%, 27.9%, and 19.5% of subjects, respectively. Prolonged aPTT and thrombocytopenia were observed three times more frequently in subjects with moderate-severe liver iron overload than in subjects with normal-mild liver iron overload (P=0.04 and 0.001, respectively).

Conclusion Most TM subjects have liver iron overload ranging from mild to severe. Prothrombin time and aPTT prolongation, as well as thrombocytopenia are easily found in TM children. There were significantly more moderate-severe liver iron deposition patients with aPTT prolongation and thrombocytopenia than normal-mild patients with these conditions. Hence, we suggest that pediatric TM patients undergo liver iron deposition evaluations and use iron chelators in an optimal manner, in order to limit the risk of bleeding. **[Paediatr Indones. 2018;58:175-9; doi: http://dx.doi.org/10.14238/pi58.4.2018.175-9]**.

Keywords: *prothrombin time; activated partial thromboplastin time; platelet; liver iron overload*

Thalassemia is an inherited blood disorder characterized by diminished production of one or more globin chains. The degree of thalassemia severity is affected by several factors, such as type of globin mutation, presence of hemoglobin variants, hereditary persistence of fetal hemoglobin (HPFH) mutation, and other mutations inside the β-globin cluster.[1]

Thalassemia major (TM) is the most severe form of thalassemia, characterized by severe anemia. Patients are totally dependent on regular blood transfusions throughout their entire lives. Red cell transfusions lead to accumulation of iron in several organs. This condition is worsened by the fact that the patient's chronic state of anemia causes increased absorption of iron from gastrointestinal tract. At the cellular level, accumulated iron increases production of reactive oxygen species, which subsequently damage the cells.[2] Therefore, iron chelation therapy plays a crucial role in the survival of TM patients, although in most conditions it is not

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sufficient to prevent iron deposition in tissues.[1,2] The liver, as the predominant iron storage organ, may be damaged earlier compared to other organs.[3]

Although a chronic hypercoagulable state has been observed in many thalassemia studies, it was mostly reported in adult subjects.[4-5] A pediatric study reported that 29.6% of thalassemia patients had bleeding manifestations, such as epistaxis and gum bleeding.[6] Loss of blood aggravates the anemic condition, and leads to increasing required volumes of blood transfusion and intensifying iron absorption in the gastrointestinal tract. Therefore, we aimed to explore liver iron deposition and hemostatic abnormalities in thalassemic children with liver iron overload, based on their laboratory values.

Methods

This cross-sectional study included 190 non-splenectomized children with TM. Spleen size was classified using Schuffner grading, which an imaginary line that connect right costal arch to spina iliaca anterior superior sinistra (SIAS). The line was divided into 8 segments equally: Schuffner 1 lies in right costal arch, Schuffner 4 lies in umbilicus, and Schuffner 8 lies in SIAS. The degree of liver iron deposition was determined using *MRI 1.5 Tesla* scanner (Siemens Avanto, Germany) with T2* gradient echo (GRE) sequence. The T2* values were analyzed using *CMRtoolsTM* software (Thalassemia-Tools, London, United Kingdom). Subjects were asked to hold their breath for 11-13 seconds (s), while scanned images were collected at 12 different echo times (1.3-23 milliseconds/ms). Liver iron overload was categorized as normal >6.3 ms, mild 2.7-6.3 ms, moderate 1.4-2.7 ms, or severe <1.4 ms.[7] The normal reference laboratory values for PT, aPTT, and platelet counts in our center were 9.8-11.2s, 31-47s, and 150,000-450,000 cells/μL, respectively. Platelet values for each subject were acquired after calculating mean platelet counts for 1 year.

Results

The mean age of subjects was 13 (SD) years. The numbers of male and female subjects were almost equal. Most subjects were diagnosed with β-thalassemia (51.1%) and β-thalassemia/HbE (45.8%). The majority of subjects were treated with deferiprone (DFP) monotherapy (65.8%), followed by combined DFP+deferasirox (DFX) (15.8%), and DFX monotherapy (9.5%). Approximately 73.2% of subjects had spleen size of Schuffner II or less, and only 10.5% of subjects had normal liver iron deposition. The PT prolongation was observed in 60% of subjects, while aPTT prolongation was observed in 27.9% of subjects. A total of 19.5% of subjects had thrombocytopenia (**Table 1**).

In order to fulfill the conditions for statistical analysis, patients were grouped into the normal-mild iron overload (group A) or the moderate-severe iron overload (group B). There was only slight difference of mean PT between group A and group B (P=0.587).

Table 1. Demographic distribution of subject characteristics

Characteristics	N=190
Mean age (SD), years	13 (2.45)
Sex, n (%)	
Male	96 (50.5)
Female	94 (49.5)
Diagnosis, n (%)	
α-thalassemia	5 (2.6)
β-thalassemia	97 (51.1)
β-thalassemia/HbE	87 (45.8)
α-β-thalassemia/HbE	1 (0.5)
Iron chelation, n (%)	
Monotherapy	
DFO	0 (0)
DFP	125 (65.8)
DFX	18 (9.5)
Combination therapy	
DFO+DFP	12 (6.3)
DFO+DFX	4 (2.1)
DFP+DFX	30 (15.8)
No chelation	1 (0.5)
Spleen size, n (%)	
Normal	34 (17.9)
Schuffner I-II	105 (55.3)
Schuffner III-IV	44 (23.2)
> Schuffner IV	7 (3.6)
Liver iron overload/Liver T2* MRI, n (%)	
Normal	20 (10.5)
Mild iron overload	55 (28.9)
Moderate iron overload	65 (34.2)
Severe iron overload	50 (26.3)
PT prolongation, n (%)	114 (60)
aPTT prolongation, n (%)	53 (27.9)
Thrombocytopenia, n (%)	37 (19.5)

DFO=deferoxamine, DFP= deferiprone, DFX= deferasirox

Interestingly, mean aPTT in group B [44.94 (6.66) s] was significantly longer than in group A [41.68 (4.92) s] (P=0.036). A significant difference was also observed in mean platelet counts between groups, with a mean difference of 59,337 cells/μL (P=0.001) (**Table 2**).

Table 3 shows that PT prolongation was observed in around 60% of subjects in both groups (P=0.915). However, aPTT prolongation was significantly higher in group B (37.2%) than in group A (13.6%); (P=0.04) (**Table 4**).

Table 5 shows that platelet counts were also significantly different between the liver iron overload groups. Thrombocytopenia was more common in group B (27.2%) compared to group A (8.1%); (P=0.001).

Discussion

In our study, PT prolongation was observed in 60% of subjects. Prothrombin time is a test to determine deficiency of clotting factors, which are involved in the extrinsic coagulation pathway, including factors II, V, VII, and X. Clotting factors, with the exception of factor VIII, are primarily synthesized in the liver. Liver dysfunction may lead to decreased bile secretion into the duodenum and subsequent impairment of vitamin K absorption. Vitamin K acts as a co-factor in the production of factors II, VII, IX, and X.6,8 Interestingly, no significant mean PT difference was observed between subjects with normal-mild iron overload and those with moderate-severe iron overload. These results suggest that production of clotting factors and bile salt secretion were impaired at an earlier stage of liver iron deposition.

In contrast, aPTT prolongation was observed in only 27.9% of subjects. Prolonged aPTT in thalassemia may be caused by liver dysfunction due to iron deposition and chronic activation of the intrinsic coagulation cascade.[6] One study suggested that chronic blood transfusions and hemolysis-induced activity of kallikrein-esterase, result in increased usage

Table 2. Mean PT, aPTT, and platelet counts in in the liver iron overload groups

Liver iron status	Mean (SD)					
	PT, s	P value	aPTT, s	P value	Platelet count, cells/μL	P value
Normal-mild iron overload (group A)	11.47 (0.89)	0.587	41.68 (4.92)	0.036	274,530 (104,215)	0.001
Moderate-severe iron overload (group B)	11.75 (1.55)		44.94 (6.66)		215,193 (90,730)	

Table 3. PT prolongation in the liver iron overload groups

Liver iron status	PT		P value
	Normal	Prolongation	
Normal-mild iron overload, n (%) (group A)	31 (40.9)	44 (59.1)	0.915
Moderate-severe iron overload, n (%) (group B)	45 (39.5)	70 (60.5)	

Table 4. aPTT prolongation in the liver iron overload groups

Liver iron status	aPTT		P value
	Normal	Prolongation	
Normal-mild iron overload, n (%) (group A)	65 (86.4)	10 (13.6)	0.04
Moderate-severe iron overload, n (%) (group B)	72 (62.8)	43 (37.2)	

Table 5. Thrombocytopenia in the liver iron overload groups

Liver iron status	Platelet counts		P value
	Normal	Thrombocytopenia	
Normal-mild iron overload, n (%) (group A)	69 (91.9)	6 (8.1)	0.01
Moderate-severe iron overload, n (%) (group B)	84 (72.8)	31 (27.2)	

of factors XII and XI.[9] Prolonged aPTT was observed in more patients with moderate-severe liver iron overload than those with normal-mild status. Factor VIII is synthesized mainly by endothelial cells, not hepatocytes.[10] As such, most patients with normal-mild liver iron overload had normal aPTT, although their PT was abnormal. It is important to note that technical factors may cause false positive results of PT and aPTT prolongation. Those factors include ratio of blood volume to citrate anticoagulant, variation in citrate concentration, and the difficulty of blood drawing in children.[11]

From those explanations, we assume that PT and aPTT prolongation were consequences of liver dysfunction and chronic activation of clotting factors. One study found that the tendencies of bleeding and thrombosis are different between children and adults. Bleeding is commonly found in children. The risk of bleeding rises with increasing age until they reach 11-16 years, which is the period of hemostatic balance between thrombophilic and anti-thrombotic. In contrast, the tendency of thrombosis is more common in adults.[6]

Thrombocytopenia is common in thalassemia patients. Two mechanisms that explain this condition are increased platelet destruction and decreased thrombopoietin (TPO) level. Increased platelet destruction is primarily caused by splenomegaly, which can be found easily in thalassemia patients due to extramedullary erythropoiesis process and extensive extravascular hemolysis.[12] In our study, 26.8% of subjects had spleen size greater than Schuffner II.

Transfusion-dependent thalassemia patients are also prone to liver iron overload, which later damages normal liver cells.[3] Chronic liver disease may also contribute in platelet destruction due to increased shear stress, fibrinolysis, and immunologic destruction mediated by anti-glycoprotein antibodies. Thrombopoietin, which is predominantly synthesized in the liver, plays important roles in megakaryocytopoiesis and platelet maturation.[13]

In conclusion, most children with TM have abnormal liver iron deposition. PT prolongation is more common compared to aPTT prolongation and thrombocytopenia. Patients with moderate-severe liver iron deposition are susceptible to aPTT prolongation and thrombocytopenia. Prolonged PT is not affected by the degree of liver iron overload. In conclusion. We suggest that children with thalassemia major undergo liver iron overload evaluation and iron chelation therapy, in order to minimize the risk of bleeding.

Conflict of interest

None declared.

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Original Article

The outcomes of childhood acute lymphoblastic leukemia with hyperleukocytosis

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Abstract

Background Hyperleukocytosis in childhood acute lymphoblastic leukemia (ALL) is an emergency in oncology. This condition showed high mortality and relapse rates, as well as low survival rate. The outcomes of this group of patients are not yet well studied.

Objective To evaluate the characteristics and outcomes of childhood acute lymphoblastic leukemia (ALL) with hyperleukocytosis.

Methods This was a retrospective cohort study. The patients were children less than 18 year of age who were diagnosed as ALL in Dr. Sardjito Hospital, Yogyakarta, from January 1, 2010 to November 30, 2016. Event-free survival rate and overall survival rate were estimated for group of patients with the white blood cell (WBC) groups 50-200 x 10^9/L and >200 x 10^9/L using the Kaplan-Meier method.

Results There were 705 children diagnosed as ALL during the study period, 129 (18%) with hyperleukocytosis and 111 of them met the inclusion criteria, consisted of 76 children in a group of WBC 50-200 x 10^9/L and 35 children in a group of WBC >200 x 10^9/L. Presentation at diagnosis: median age were 7 years (range 1 month-18 years), male was 1.5 higher than female, 92% of cases with lymphoid infiltration, 5% with CNS involvement, 40% had bleeding tendency, and 10% had clinical tumor lysis syndrome (TLS). Median WBC was 122 (range 53.4-876) x 109/L; mean Hb was 8 (SD 3) g/dL; median platelet count was 30 (range 1-221) x 10^9/L. Immunophenotyping was done in 23 patients, 5/23 (8%) was T cell. The patients in lower WBC group showed lower death (26% *vs.* 34%, P=0,389), higher two-year event-free survival (EFS) 68% *vs.* 45%, P=0.003, and overall survival (77% *vs.* 68%, P= 0.16), compared to patients in higher WBC group. Univariate and multivariate Cox regression analyses revealed that none of the variables was a significant prognostic factor for 2 years EFS or overall survival.

Conclusion The group of children with ALL and hyperleukocytosis with lower WBC at diagnoses showed better outcomes than the higher WBC. **[Paediatr Indones. 2018;58:186-91; doi: http://dx.doi.org/10.14238/pi58.4.2018.186-91]**.

Keywords: *hyperleukocytosis; white blood cell; survival rate; childhood acute lymphoblastic leukemia; ALL*

Acute lymphoblastic leukemia (ALL) is the most common type of leukemia in children. This is a condition were hematopoietic cells proliferate and accumulate excessively.[1] The epidemiological data of childhood ALL differs among countries, this may due to genetic predisposing factors, exposure to infectious diseases, and other environmental factors.[2] The incidence of childhood ALL is 3.6 cases out of 100,000 children, and 1.4 cases out of the entire population per year.[3] The incidence of childhood ALL is higher in North and West Europe, North America, and Oceania, compared to Asia and Africa.[1] In Europe, there were 46.7 new cases per one million population in 2009.[4] However, in Asian countries new cases were estimated to be 54,000 new cases per year.[5] A study in Dr. Sardjito Hospital, Yogyakarta, found that childhood ALL comprised 68.9% of all leukemia cases in children who were diagnosed during 1998-2009.[6]

Hyperleukocytosis was defined as WBC more than 50-100 x 10^9/L.[7,8] This presentation is related to

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poor outcomes.[9] The most common complications are metabolic, such as hyperphosphatemia, hypocalcemia, hyperkalemia, hyperuricemia, and hemodialysis (33.3%), as well as neurological effects, such as seizures, bleeding, and respiratory complications in 8.3%.[10] Hyperleukocytosis complications lead to a high mortality rate of 20% and a low event-free survival rate of 63.3% vs. 100% for >100 x 10^9/L *vs.* < 100 x 109/L.[11,12] The prognosis of childhood ALL with hyperleukocytosis worse than those without hyperleukocytosis. Some studies revealed that hyperleukocytosis increased the risk of poor outcomes by 12 times, including high relapse and mortality rates and low survival rate.[10,13] The poor outcome such as relapse rate was significantly correlated with high leukocyte count. The WBC of > 300 x 10^9/L resulted in shorter survival, highest relapse rate, and highest mortality rate caused by chemotherapy. In contrast, WBC of 200-300 x 10^9/L had the highest survival rate and lowest relapse rate for unknown reasons.10 In comparison, WBC < 100 x10^9/L *vs.* >100 x 10^9/L resulted in a 4 year survival rate of 79 (SD 4) % *vs.* 52 (SD 8) %, respectively (P=0.0001), while WBC 100-200 x 10^9/L *vs.* > 200 x 10^9/L resulted in a survival rate of 4 years [64 (SD 10) % *vs.* 34 (SD 14)%, respectively (P=0.04)].[12]

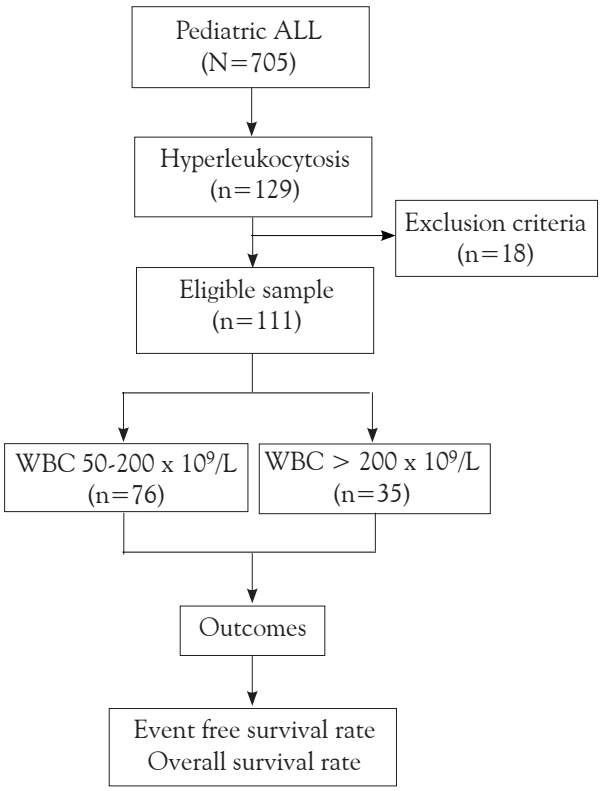

Figure 1. Study flow diagram

Methods

This retrospective cohort study was conducted in Dr. Sardjito Hospital, Yogyakarta. Subjects were pediatric ALL patients, aged 1 month-18 years with hyperleukocytosis (WBC > 50 x10^9/L) at the time of diagnosis, and treated in the Oncology Ward during the periode of January 1, 2010 to November 30, 2016. The diagnosis of ALL was based on leukemic cell morphology identification under light microscope, cytochemistry, and immunophenotyping of marrow aspirates. Patients with incomplete medical record were excluded from this study. Flow of the study is shown in **Figure 1**.

The event free survival (EFS) and overall survival (OS) were analyzed using Kaplan-Meier log-rank test utilized *SPSS version 20* program. The events in this study were death (all systemic organs stopped functioning and marked by brain stem death); abandonment (discontinuation of treatment more than 2 weeks or refused treatment); relapse, defined as (1) isolated bone marrow relapse: blast cells > 25% in bone marrow puncture or biopsy without central nervous system (CNS) and testicular involvement after remission already reached, (2) isolated CNS relapse: positive cytomorphology and WBC > 5/μL and CNS signs and symptoms, (3) isolated testicular relapse: leukemic infiltration in the testicle confirmed by biopsy, and (4) combined relapse: M2 or M3 after remission, with CNS or testicular involvement and disease resistance (blast > 5% from bone marrow aspiration at the end of induction phase). Bone marrow remission status was classified as M2 or M3 if the blast in bone marrow was 5-25% or > 25%, respectively.[13]

The survival analysis was conducted for two WBC groups: those with 50-200 x 10^9/L and those with > 200 x 10^9/L, following the studies already done.[10,12] Potential prognostic factors were analyzed by bivariate with Cox regression model; variables with P values < 0.25 at univariate analysis were included

in the multivariate analysis. A P value of < 0.05 was considered to be statistically significant. Age and immunophenotype stratification in bivariate and multivariate analyses were compared to the group with good prognosis, namely, patients aged 1-10 years and B cell immunophenotype.

Results

During the study period, there were 705 childhood ALL patients, 129 (18.3%) had hyperleukocytosis, however, 18 were excluded due to incomplete medical records. Subjects were divided into two groups, based on WBC of 50-200 x 109/L or >200 x 109/L. The characteristics of subjects are described in **Table 1** and case mortality by WBC group is shown in **Table 2**. The two-year EFS for the WBC 50-200 x 10^9/L and > 200 x 10^9/L groups were 68% and 45%, respectively (HR 2.3; 95%CI 1.30 to 4.12; P=0.003). The OS rates of the WBC groups were 77% and 68%, respectively (HR 1.6; 95%CI 0.81 to 3.41; P=0.16).

Kaplan-Meier analysis for EFS and OS are presented in **Figures 2** and **3**. Bivariate analysis was performed using Cox regression method to see the effect of independent variables on EFS

Table 1. Subjects' characteristics (N= 111)

Characteristics	WBC, x 10^9/L		Total	P value
	50-200 (n= 76)	>200 (n= 35)		
Age at the time of diagnosis, n (%)	76 (68.47)	35 (31.53)	111	0.082
<1 year	2 (2)	3 (3)	5 (4.50)	
1-10 year[a]	46 (41)	15 (14)	61 (54.95)	
>10 year	28 (25)	17 (15)	45 (40.54)	
Median age (range), year	7.35 (0.13-18.07)			
Sex, n (%)	76 (68.47)	35 (31.53)	111	0.136
Male	43 (39)	25 (22)	68 (61.27)	
Female	33 (30)	10 (9)	43 (38.73)	
Infiltration of lymphoid tissue, n (%)	76 (68.47)	35 (31.53)	111	0.229
No, n (%)	7 (6)	1 (1)	8 (7.20)	
Yes, n (%)	69 (62)	34 (31)	103 (92.80)	
Immunophenotype, n (%)	13 (56.52)	10 (43.48)	23	0.008
T cell	1 (4)	4 (18)	5 (8.30)	
B cell[b]	11 (48)	3 (13)	7 (58.30)	
Mixed	1 (4)	3 (13)	4 (33.30)	
Median leukocyte count (range), 10^9/L	122 (53.4-876)			
Hb, n (%)	76 (68.47)	35 (31.53)	111	0.123
>6 g/dL	55 (50)	30 (27)	85 (76.58)	
<6 g/dL	21 (19)	5 (4)	26 (23.42)	
Mean Hb (SD), g/dL	8.04 (2.58)			
Platelet count, n (%)	76 (68.47)	35 (31.53)	111	0.012
> 20 x 10^9/L	50 (45)	31 (28)	81 (72.97)	
< 20 x 10^9/L	26 (23)	4 (4)	30 (27.03)	
Median (range), 10^9/L	30 (1-221)			
CNS involvement, n (%)	45 (61.64)	28 (38,36)	73	0.572
No	42 (58)	27 (37)	69 (94.52)	
Yes	3 (4)	1 (1)	4 (5.48)	
Bleeding evidence, n (%)	76 (68.47)	35 (31.53)	111	0.434
No	44 (40)	23 (20)	67 (60.36)	
Yes	32 (29)	12 (11)	44 (39.64)	
Clinical TLS, n (%)	76 (68.47)	35 (31.53)	111	0.002
No	73 (66)	27 (24)	100 (90.09)	
Yes	3 (3)	8 (7)	11 (9.91)	

[a]Good prognosis for age stratification, [b]Good prognosis for immunophenotype stratification

Table 2. Outcomes for two-year EFS by WBC group

WBC (x 10^9/L)	Outcomes						Total
	Survived					Died	
	Without event	Disease resistance	Relapse	Drop out	Total		
50-200, n (%)	49 (64.47)	3 (3.95)	1 (1.31)	3 (3.95)	56	20 (26.31)	76
>200, n (%)	14 (40)	5 (14.28)	2 (5.71)	2 (5.71)	23	12 (34.28)	35
Total	63	8	3	5	79	32	111
P value							0.389

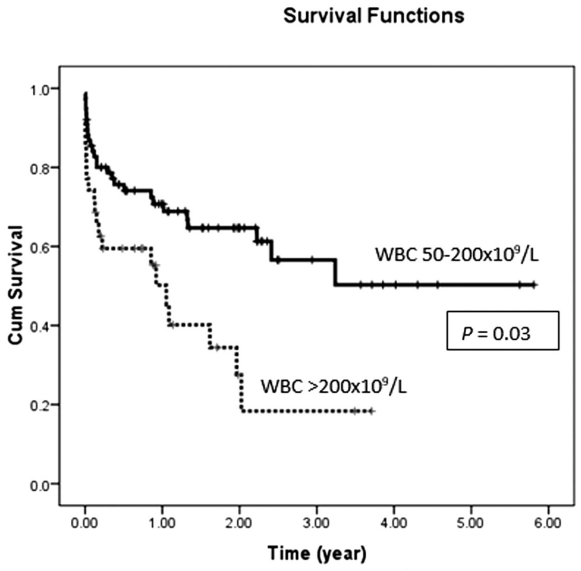

Figure 2. Event-free survival rate

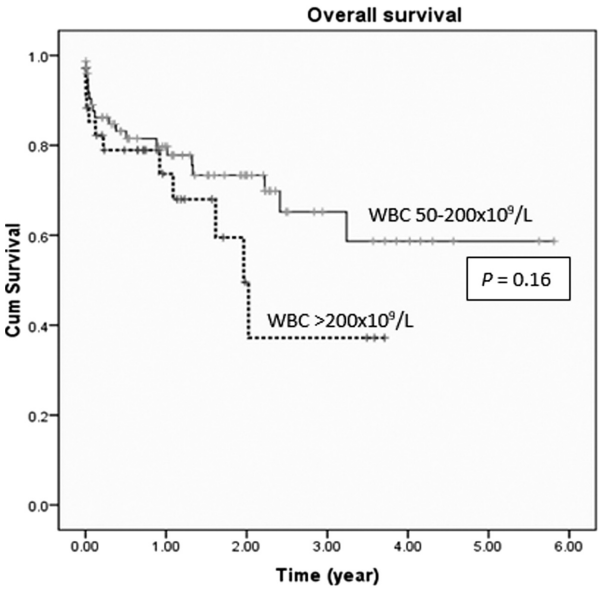

Figure 3. Kaplan-Meier curve for overall survival rate

Table 3. Multivariate analysis for prognostic factors of EFS

Variables	EFS		
	P	HR	95%CI
Age stratification	0.073	14.72	0.78 to 277.04
Hb stratification	0.98	0,0004	-
Leukocyte stratification	0.058	18.64	0.91 to 383.29
Platelet stratification	0.98	0.0001	-
Immunophenotype	0.37	0.29	0.02 to 4.20
CNS Involvement	0.99	0.00023	-

was shown to not be a significant prognostic factor for OS. The multivariate analysis results are shown in **Table 3**.

Discussion

The incidence of hyperleukocytosis and subject characteristics in this study were similar to others, in terms of sex, mean age, and presence of lymphoid infiltration.[10,12,14] Male sex and lymphoid infiltration had significant associations with hyperleukocytosis.[10,15,16] In addition, the age group of 1-10 years had a significant association with the incidence of WBC count > 200 x 10^9/L.[10]

The differences in our study from other studies were imunophenotype B-cell as the most common immunophenotype and only a few patients had symptoms of TLS. But only 23/111 (21%) patients underwent immunophenotyping. Only 10% of patients had TLS symptoms, but objective parameter such as laboratory findings were not explored in our study. In this study, there was significant differences between group of WBC count 50-200 x 10^9/L and > 200 x 10^9/L in term of platelet stratification (P=0.012), immunophenotype (P=0.008) and

clinical TLS (P=0.002) but after continuing with multivariate analysis, there was no significant prognostic factor for EFS. However, previous studies reported that hyperleukocytosis had significant relationships with T cell ALL and CNS leukemia.[12,14] Because of our small sample sizes, we cannot conclude that T cell phenotype or TLS were associated with hyperleukocytosis. Nor can we suggest that race or geography may play a role in our findings. In previous studies, geographic area were suggested to differ in genetics and environmental exposure that may lead to cancer risk.[2,15] As such, ALL is likely to be caused by multiple factors.

We found that the two-year EFS was significantly different between the WBC 50-200 x 10^9/L and >200x109/L groups [68% *vs.* 45%, respectively, (HR 2.3; 95%CI 0.88 to 3.56; P=0.003)]. However, the two-year OS was not significantly different between the two leukocyte groups [77% *vs.* 68%, respectively (HR 1.69; 95%CI 1.49 to 2.43; P=0.16)]. The EFS and OS results were similar to those of previous studies.[10,12,13] The four-year EFS in another studi was 52 (SD 8) % for patients with WBC > 100 x 10^9/L *vs.* 79 (SD 4) % for patients with WBC < 100 x 10^9/L (P< 0.0001). In addition, high leukocyte count and massive splenomegaly were poor prognostic factors for EFS.[12] Another study showed that the three-year EFS and OS for childhood ALL with hyperleukocytosis were 75% and 81.2%, respectively.[10] Furthermore, Yang et al. in 2016 reported a difference in EFS, with the shortest EFS in the WBC >300 x 10^9/L group (P=0.006).[14] We suspected that higher WBC count may lead to poor EFS, because we observed a higher mortality rate in the WBC > 200 x 10^9/L group (34%) than in the WBC 50-200 x 10^9/L group (26%), but the P value shows not significant (P= 0.389).

We found that 4.5% of our subjects abandoned treatment and 29% died. The mortality rate of the WBC 50-200 x 10^9/L was 26% (20/76), while that of patients with WBC > 200 x 10^9/L group was 34% (12/35). Similarly, other studies showed that the higher the WBC at the time of diagnosis, the poorer the prognosis.[8,14] A previous study in Indonesia in 1997-2002 reported that 35% of children drop out from ALL treatment, 23% experienced treatment-related death and 20% had an overall event-free survival rate.[17] Our findings may differ, as most of our patients had insurance for treatment. A lack of financial resources, medical facilities, or social support services in the previous study may have contributed to discontinued treatment.[18]

In conclusion, higher WBC count at the time of diagnosis may lead to poorer prognosis in pediatric ALL patients with hyperleukocytosis. However, a longer study period is needed as this study was undertaken to obtain primary data on hyperlukocytosis in childhood ALL in developing countries, like Indonesia.

Conflict of Interest

None declared.

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Original Article

Age and HIV stage at initiation of highly active antiretroviral therapy determine non-reversal stunting at 3 years of treatment

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Abstract

Background Highly active antiretroviral therapy (HAART) has been reported to improve growth, especially in the first 2 years of treatment. It is not clear whether catch up growth is maintained after 2 years of HAART.

Objective To assess growth in stunted children with HIV after 3 years of HAART and analyze possible risk factors for non-reversal of stunting.

Methods This study was done from May 2016 to April 2017 to follow children with HIV who started HAART between January 2009 and April 2014, and continued for 3 years. Inclusion criteria were children with HIV, aged < 18 years, compliance to the regimen, and stunting. Exclusion criteria were patients lost to follow up or who died prior to 3 years of HAART. Non-reversal of stunting was defined as HAZ ≤ -2SD after 3 years of HAART. Possible risk factors for non-reversal were analyzed using Chi-square test with P<0.05, as well as risk ratio (RR) and 95% confidence intervals (CI).

Results Of 150 HIV-infected pediatric patients, 115 were on HAART and 55 (47.8%) were stunted at HAART initiation. Of the 55 stunted and HAART-treated children, 31 (56.4%) were male. Baseline median age was 3.6 years (interquartile range 0.37-8.48). Non-reversal occurred in 32 (58.2%) subjects. Multivariate Cox regression model analysis showed predictors of non-reversal after 3 years of HAART to be age >2 years (RR 16.05; 95%CI 2.89 to 89.02; P=0.002) and HIV stage III-IV (RR 8.93; 95%CI 1.47 to 54.37; P=0.017).

Conclusion The HAART initiation at age >2 years and HIV clinical stage III-IV at diagnosis are risk factors for non-reversal of stunting after 3 years of HAART. **[Paediatr Indones. 2018;58:180-5; doi: http://dx.doi.org/10.14238/pi58.4.2018.180-5]**.

Keywords: *HIV; stunted; HAART; three-year observation; non-reversal*

In the last two decades, Indonesia has seen a concentrated HIV epidemic expand into a generalized one, as a result of the growing number of women of childbearing age who are infected with HIV.[1,2] The cumulative number of children under 15 years of age with HIV in Indonesia from 1987 to 2014 was reported to be as many as 1,647, of whom 1,206 were under the age of five years.[3] *The World Health Organization* (WHO) noted that only 21-25% of children infected with HIV received antiretroviral therapy.[4] Although HAART improves the survival of children with HIV, optimizing health and quality of life of pediatric survivors remains a major challenge.[5] Without effective treatment, an estimated one-third of children with HIV die in the first year of life, and about half die in the second year.[6]

Several studies reported growth improvements in children with HIV after HAART initiation. Mwiru *et al.* found that children with HIV showed

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improvement in metabolism in the first 6 months of HAART.[7] Similarly, Suryani *et al.*[8] observed growth improvement for 12 months, and Sari et al. showed growth improvement at 24 months of HAART.[9]

Although studies have reported growth and nutritional care improvements within 2 years of HAART, other studies noted that stunting may occur after such time.[7,10,11] A study of 225 HIV-infected children found a probability of 58% for growth reversal among 179 subjects with growth failure at entry, while the rest remained in growth failure. Age, sex, and clinical category at the time of ART initiation were not risk factors for reversal.[12] Therefore, we aimed to assess if non-reversal of stunting occurred in children with HIV after three years of HAART and to examine risk factors for non-reversal of stunting.

Methods

The study was a part of a prospective cohort study which followed children with HIV who initiated HAART between January 2009 and April 2014, in particular, those with stunting. The study was conducted from May 2016 until April 2017 in the Allergy Immunology Division, Department of Child Health, Sanglah Hospital, Denpasar, Bali. Subjects were part of the eligible population who met the following inclusion criteria. Children with HIV aged < 18 years, good HAART compliance, stunted at the time of HIV diagnosis, and whose parents were willing to participate. Exclusion criteria were lost to follow up or died during observation. In addition to HAART, all children received daily zinc supplementation.

We collected subjects' age, sex, weight, height, height-for-age *z*-score (HAZ) to determine stunting, recent opportunistic infection, recent anemia, CD4+, nutritional status, family economic status, HIV clinical stage at the start of ART, and 3 years of data to determine linear growth pattern, and risk factors for non-reversal of stunting. All data were collected from the subjects' medical records. The variables were defined as follows:

- Stunted: HAZ <-2SD according to the WHO Child Growth Standard.[13] Stunting was determined at the time of diagnosis and calculated using the *2005 WHO Anthro and Anthroplus software*.
- Opportunistic infection: an infection in a person with poor immunity, which would normally not cause infections in those with normal immunity.[14]
- Anemia: determined based on the WHO criteria for HIV clinical stage 3, in which hemoglobin was < 8 g/dL.[15]
- Nutritional status: determined using the Waterlow formula, in which actual body weight was divided by ideal body weight. Ideal body weight was determined using the *WHO Growth Chart* for body weight over height.[16]
- Family economic status: the economic ability to meet the needs of all family members. Our subjects were assumed to have similar economic status, since all subjects used government health care insurance.
- HIV clinical stage: determined based on the WHO clinical stage category.[15]
- Non-reversal of stunting: HAZ <-2SD after 3 years of HAART.

Characteristics of subjects were presented descriptively in tables for the variables of age, sex, and HIV clinical stage, as well as tuberculosis, anemia, chronic diarrhea, CD4+, and nutritional status. Linear growth patterns during 3 years of HAART was presented as a graph. We calculated the proportion of non-reversal of stunting in our subjects. Possible risk factors for non-reversal were analyzed by Chi-square test. Covariates were considered for inclusion in the multivariate model if one or more categories exhibited a P value <0.1 in the bivariate model and were retained if 1 or more categories exhibited a P value <0.05 in the adjusted model.[17] Risk ratio (RR) of associated factors was determined with 95% CI. Analyses were done with *SPSS version 18 software*. This study was approved by the Medical Ethics Committee of Universitas Udayana/Sanglah Hospital, Denpasar.

Results

A total of 150 children were in the data cohort. Of these, 118 children were alive and 115 children had received HAART. At baseline, 55 (47.8%) children were stunted at HAART initiation, hence, they were included in our study. Only 23 (41.8%) subjects achieved growth reversal after 3 years of HAART

Table 1. Demographic and clinical characteristics of stunted, HIV-infected children at the time of HAART initiation

Clinical characteristics	(N=55)
Sex, n (%)	
Male	31 (56.4)
Age at baseline, n (%)	
≤ 2 year	12 (21.8)
> 2 year	43 (78.2)
Anemia at baseline, n (%)	
Yes	8 (14.5)
No	47 (85.5)
Nutritional state at baseline, n (%)	
Severe-moderate malnutrition	38 (69.1)
Well-nourished	17 (30.9)
Chronic diarrhea at baseline, n (%)	
Yes	5 (9.1)
No	50 (90.9)
Tuberculosis at baseline, n (%)	
Yes	21 (38.2)
No	34 (61.8)
CD4+ level, n (%)	
< 15% or < 200 cells/μL	39 (70.9)
≥ 15% or ≥ 200 cells/ μL	16 (29.1)
HIV stage at baseline, n (%)	
Stage I-II	8 (14.5)
Stage III-IV	47 (85.4)
Mean HAZ (SD)	-3.27 (0.9)
Stunting after 3 years of HAART initiation, n (%)	
Reversal	23 (41.8)
Non-reversal	32 (58.2)

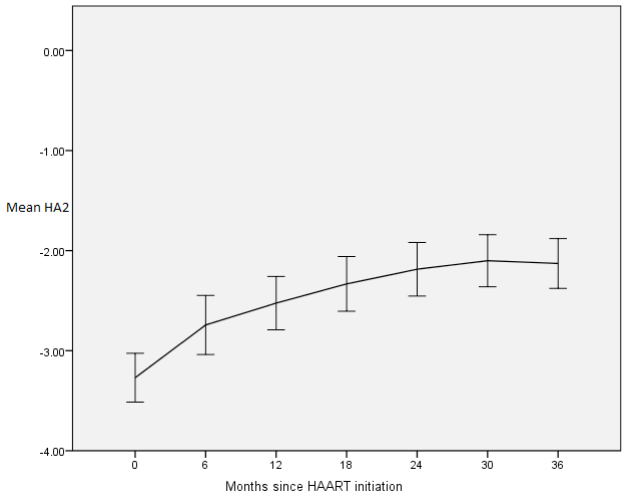
initiation. Characteristics of the 55 subjects are shown in **Table 1**.

The mean HAZs during the follow up period are shown in **Figure 1**. Upon on HAART initiation, mean HAZ improved for the first 6 months, then stabilized with only minimal improvements in linear growth over the 3 years of HAART. The trajectory of HAZ over the 3-year observation tended to plateau.

Table 2 shows the factors associated with non-reversal of stunting. Multivariate Cox regression analysis of the 55 children who were stunted at baseline revealed that the strongest predictors for non-reversal of stunting were age>2 years (RR 16.05; 95%CI 2.89 to 89.02; P=0.002) and HIV stage III-IV at HAART initiation (RR 8.93; 95%CI 1.47 to 54.37; P=0.017).

Discussion

Stunting is defined as height for age below minus 2 SD, according to *The WHO Child Growth Standard*. Stunting indicates a failure to achieve potential linear growth as a result of suboptimal health and/or nutritional conditions.[13] Children with HIV infection have a higher risk of stunting compared to uninfected children. In Tanzania, 36.6% of children infected with



	0	6	12	18	24	30	36
Mean HAZ	-3.27	-2.74	-2.52	-2.33	-2.19	-2.10	-2.13
Mean change	0	0.53	0.22	0.19	0.14	0.09	-0.03
% Stunted	100	81.8	74.5	61.8	65.5	61.8	58.2

Figure 1. Mean HAZ over the 3-year follow up period after HAART initiation

Table 2. Predictors of non-reversal of stunting

Characteristics	Bivariate[a]		Multivariate[b]	
	Risk ratio (95%CI)	P value	Risk ratio (95%CI)	P value[c]
Age >2 years	2.76 (1.64-4.64)	0.001	16.05 (2.89-89.02)	0.002
Anemia	1.41 (0.87-2.28)	0.225	NA	
Severe-moderate malnutrition	1.28 (0.73-2.26)	0.262	NA	
CD4+ level	0.64 (0.35-1.17)	0.138	NA	
Chronic diarrhea	1.07 (0.50-2.28)	0.623	NA	
Tuberculosis	1.33 (0.84-2.09)	0.176	NA	
HIV stage III-IV	2.07 (1.19-3.60)	0.048	8.93 (1.47-54.37)	0.017

[a]By Chi-square analysis; [b]By Cox regression analysis; [c]Only characteristics with P value ≤ 0.1 in bivariate analysis were included in the multivariate analysis

HIV were stunted, compared to 21.8% of children who were not infected with HIV.18 McDonald *et al.* studied 2,387 infants born from HIV-infected mothers for 21 months in Tanzania. They found that stunting occurred in 8.7 months of observation, and the risk factors for stunting in these infants included low maternal education, poverty, low birth weight, HIV infection, and male gender.[18] The age of 6 to 24 months is the most critical period for linear growth in children, and the time of peak incidence of stunting in children in developing countries.[19] In this study from a database of 150 children with HIV infection, we found 36.7% children with stunting at time of HAART initiation. Similarly, Aurpibul *et al.*[10] reported 42% in Thailand, but an African study found an even higher proportion of children with stunting, 76.3%.[12]

The goal of administering HAART to HIV-infected children is to prolong life and ensure that they achieve sustained growth and development.[19] In a Dar es Salaam study in 2004, HIV-positive children without ART therapy had a higher proportion of stunting.[14] Prendergast *et al.* assumed that the phenomenon of stunting occurred due to a state of low-grade inflammation, due to HIV infection as a result of enteropathy.[20] HIV infection may result in stunting, despite having good nutrition and a normally functioning endocrine system. Several studies described positive short-term responses of HAZ to ART.[21] Kabue *et al.* found that administering ART resulted in improvement in HAZ in all subjects who had at least 6 months Of follow-up, and again at 12 months follow-up, respectively.[22] Similar results were found by Suryani *et al.*,[8] who observed children with HIV infection for 12 months, and Sari *et al.*,[9] who observed children with HIV infection for 24 months. However, few studies reported a growth response to ART beyond 24 months.[22] Sutcliffe *et al.* reported that after starting ART, nutritional status improved in both weight and height, but even after two years of ART, approximately 50% of children remained stunted.[23] With a longer period of observation, we found that in 58.2% of children, stunting was not reversed, despite receiving ART for 3 years. Mwiru *et al.* also reported that after ART initiation, child anthropometry *z*-score profiles improved, however, HAZ scores did not reach the normal value even after 6 years of follow up.[7]

Some factors were associated with reversal of growth failure. Aurpibul et al. found that a nevirapine-based regimen was a significant predictor of reversal of growth failure after initiating ART.[12] In our study, all subjects received WHO-recommended first-line, antiretroviral drugs in the form of two NRTIs and one NNRTI, including zidovudine, lamivudine and nevirapine. Children with HIV infection may have underlying chronic conditions or opportunistic infections, including anemia, tuberculosis, and chronic diarrhea, all of which can result in growth faltering by inadequate food intake and increased resting energy expenditure.[22] Diarrhea is the most common chronic condition among HIV-infected children, causing acute weight loss through water and electrolyte loss, leading to poor growth.14 Diarrhea was associated with weight-for-age *z*-scores (WAZ) <-2SD.[14] However, we found that anemia, tuberculosis, chronic diarrhea, and malnutrition at the time of ART initiation were not associated with non-reversal of stunting in this population. Similarly, Mwiru *et al.* found that anemia, tuberculosis, and chronic diarrhea were not associated with growth outcome.[7]

Age >2 years at HAART initiation was a significnt predictor of non-reversal of stunting in our study, similar

to findings of Mwiru *et al.*[7] Better growth outcomes among younger children could be related to the duration of infection, allowing them to more effectively absorb nutrients after viral suppression by ART. The longer duration of HIV infection in older children may further require more time to reserve the damage, implying a more prolonged metabolic cost.[7,24]

Sunguya *et al.* found that advanced HIV stage was a risk factor for stunting. At advanced stages, a child may succumb to poor linear growth, even with adequate food availability, due to frequent opportunistic infections. These severe opportunistic infections may lead to increased energy expenditure at rest.[14] This result was similar to our finding that stage 3 and 4 HIV at HAART initiation was a significant risk factor for non-reversal of stunting. In contrast, Diniz et al. reported a greater height catch-up after starting HAART in subjects with clinically advanced disease at baseline.[25]

A limitation of our study was not including parental, family, or caregiver data, as another study found that family situation was related to growth.26 In conclusion HAART initiation at age >2 years and HIV clinical stage III-IV at diagnosis are risk factors for non-reversal of stunting after 3 years of HAART.

Conflict of interest

None declared.

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Case Report

Long term follow-up of multidrug resistant tuberculosis in a pubertal child

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Increasing awareness of the rising global rates of multidrug-resistant tuberculosis (MDR-TB) has led to a concerted international effort to confront this disease. Nonetheless, despite cure rates >80% in some programs, MDR-TB patients tend to have chronic disease and require prolonged therapy.[1-3] Little is known about the long-term results and follow-up of patients with MDR-TB, include the recurrence rate and chronic disability in patients who have recovered from TB.[4]

There are many side effects and adverse reactions to drugs can occur during MDR-TB treatment. These could be physical and or psychological, as well as reversible or irreversible. Treatment of MDR-TB requires a combination regimen, consists of second and third-line anti-tuberculosis drugs which more toxic than the first-line drugs. Additionally, MDR-TB treatment requires a long duration of treatment (18-24 months) and causes discomfort in the patient.[5] In a cohort of 60 patients treated for MDR-TB, the most common side effects included gastritis (100%), dermatological disorders (43%), and peripheral neuropathy (16.7).[6] While in a cohort of 75 patients, the incidence of depression, anxiety, and psychosis for MDR-TB treatments was 13.3%, 12.0%, and 12.0%, respectively.[7]

Aggressive and effective management are needed so the patient can tolerate the treatment and remain adhere to the treatment.[8] Long-term follow-up is required for the rehabilitation of disorders due to psychosocial sequelae. As such, psychosocial support can be benefit pediatric MDR-TB patients.

Here, we present a case report on a two-year follow-up of a pubertal child with MDR-TB, focusing on medical aspects and her development. **[Paediatr Indones. 2018;58:198-204; doi: http://dx.doi.org/10.14238/pi58.4.2018.198-204]**.

Keywords: *MDR-TB; pubertal children; long-term follow-up*

The Case

A 13-year-old girl came to Dr. Soetomo Hospital emergency room complaining of shortness of breath, which worsened in two days prior to hospital admission. Other complaints were cough, sub-febrile, sweating, decrease in appetite since two years before admission, and no weight gain since three years before admission. Sixteen months before admission, she has been treated as pulmonary tuberculosis in Jombang

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District Hospital. She received four anti-tuberculous drugs (rifampicin 300 mg, isoniazid/INH 200 mg, and pyrazinamide 600 mg). After completed the course of treatment, the patient stopped taking the medications, despite her condition was worsened. The history of TB contact was her father. He has been diagnosed as pulmonary tuberculosis ten months before the patient getting sick. He already completed his tuberculosis treatment. Our patient had been received BCG immunization.

Physical examination on admission revealed bodyweight was 20 kg (P <5, CDC NCHS, 2000),[9] height 136.5 cm (P25-50, CDC NCHS , 2000),[9] percent ideal body weight (IBW) 62%,[9] head circumference 51 cm (<0 SD, Nellhaus, 1968).[10] Nasal flare, sternocleidomastoid muscles contraction, enlarged lymph nodes of the neck, lag of left chest movement, and intercostal retractions were found. Percussion of the chest wall revealed resonant sound on right chest and dim sound on left chest. There were decreased vesicular breath sounds on the left chest, and coarse crackles in both the right and left chest.

Laboratory results on admission were leukocytes 9,300/uL, hemoglobin 11.7 g/dL, blood gas analysis revealed pH 7.37, $PaCO_2$ 30.1mmHg, PaO2 164.4, HCO3 28.4 mmol/L, TCO2 29.3 mmol/L, BE 6.2 mmol/L, and SO2 95.7%. Chest X-ray showed fibro-infiltrate on right lung field, left lung damage, and tracheal deviation to left side. Subsequent examination in the ward revealed positive sputum acid-fast stain, multi-slice computed tomography (MSCT) scan of the chest revealed fibroinfiltrate on right lung, multiple bullae on left lung, pleural thickening on upper right and whole left lung. GeneXpert examination revealed positive for *Mycobacterium tuberculosis* (MTB) resistant to rifampicin.

Patient was treated as MDR-TB. The first 6-month regimen included kanamycin, ethionamide, cycloserine, levofloxacin, pyrazinamide, and ethambutol, and followed by 18 months treatment with ethionamide, cycloserine, levofloxacin, pyrazinamide, and ethambutol. She experienced adverse effects of anti-tuberculous drugs include vomiting, dizziness, numbness, glare at eyes, sleep disorders, emotional disorders, and hyperuricemia. Vomiting and dizziness resolved spontaneously after the end of treatment. Numbness subsided after patient received pyridoxine. Glare testing has been consulted to Ophthalmology Department that revealed no abnormalities was found and glare at eyes were disappeared after the treatment had been completed.

As a result of pulmonary TB, patient experienced

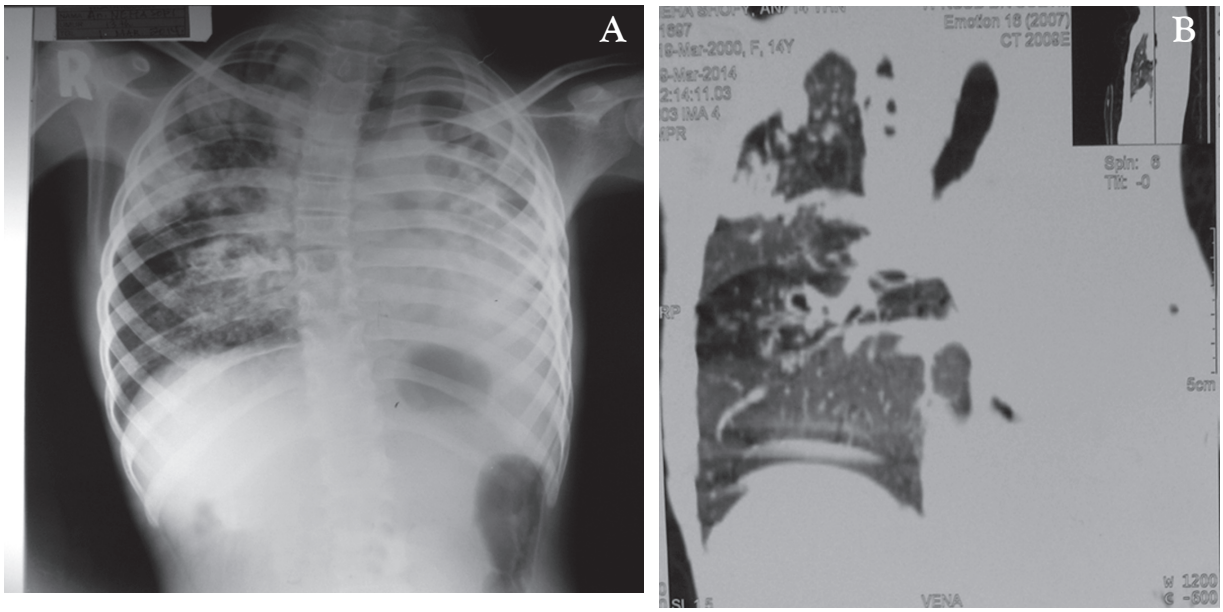

Figure 1. A. Patient chest X-rays on Emergency Department of Dr. Soetomo Hospital revealed the presence of infilitrate on right lung, tracheal deviation to the left, and left lung damage. B. MSCT scan also showed left lung damage.

severe pulmonary parenchymal damage in her left lung. Patient was easily fatigued while walking. The results of the pulmonary function tests included forced expiratory volume-1/forced vital capacity (FEV1/FVC) 98.9% predictive value and FVC 51.1%, indicate presence of restrictive lung abnormalities. The lung damage also had caused pulmonary hypertension. Echocardiography revealed pressure gradient (PG) value between the right atrium and pulmonary artery was 52 mmHg, therefore, the patient was given sildenafil citrate. Damage to the lungs had led scoliosis, hence, patient underwent rehabilitation, using a Milwaukee Brace for scoliosis correction, as well as training, in the form of exercises for scoliosis, breathing, and endurance.

Monthly evaluation of sputum acid-fast smear staining was negative and sputum culture revealed no MTB growth on the 1st to 24th month of evaluation.

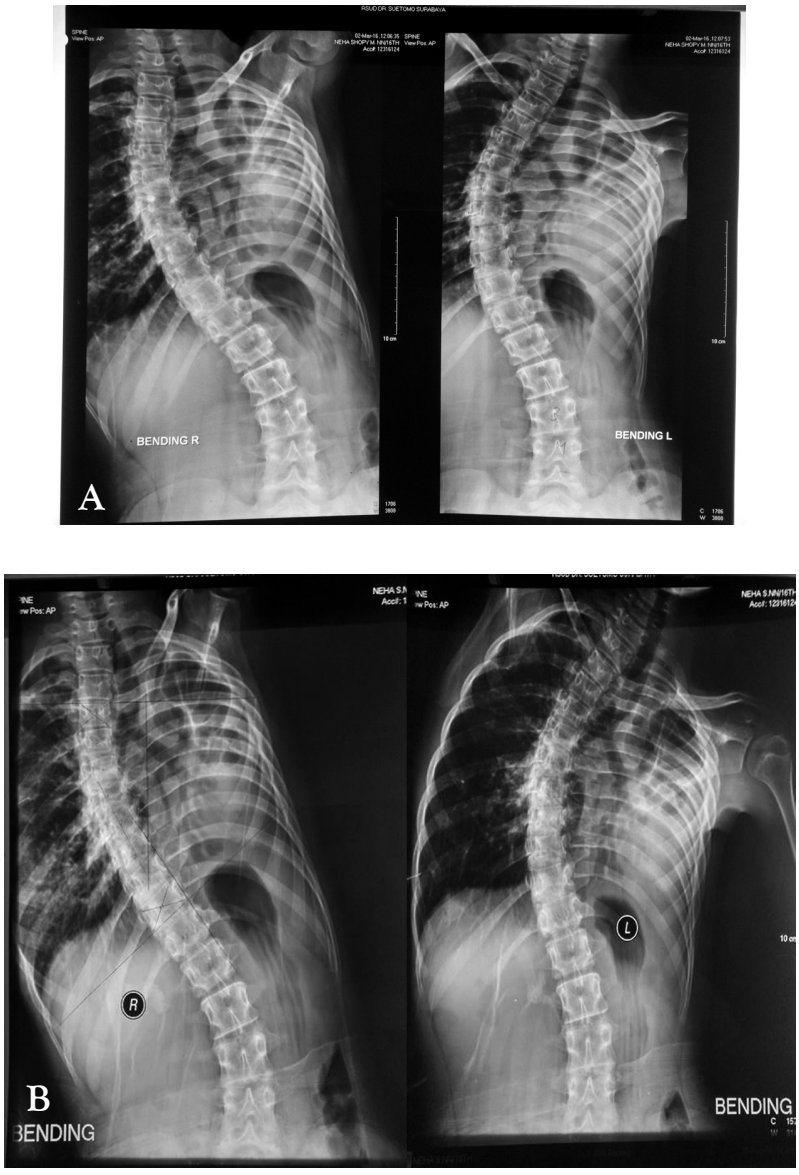

Figure 2. A. Radiological examination of scoliosis when the patient first visited the Psychiatric and Rehabilitation Department revealed double curve scoliosis and Cob angle was 53º. B. Six-month evaluation after the patient underwent full-time bracing (Milwaukee brace) and scoliosis exercises revealed single curve scoliosis and Cob angle was 50º.

Laboratory results for monitoring the adverse and side effects of drugs revealed normal results of complete blood count, normal serum electrolyte, normal kidney function, and normal liver function. However there was increasing in uric acid and improved by allopurinol therapy.

Had been completed the treatment for two years, her bodyweight was 30 kg (P <5, CDC NCHS, 2000),[9] height 143 cm (P <5, CDC NCHS, 2000),[9] and nutritional status poor nutrition (% IBW 54%)[9] and stunted. Mid-parental height (MPH) of the patient ranged from 142.5 to 159.5 cm. Patient have reached Tanner stage A2M2P2 and menarche after MDR-TB treatment was completed at the age of 15 years.

During the course of treatment, she experienced emotional and behavioral disorders. Her family reported she was sensitive and grumpy. She had been screened for behavioral disorders using *Pediatric Symptom Checklist 17* (PSC-17).[11] Her initial PSC-17 evaluation revealed internalization 8, externalization 12, attention 8, and total score 28. These mean that she had behavioral disorders. After completing the MDR-TB treatment, PSC-17 scores was improved. Internalization score was 5, externalization score was 6, attention score was 4, and total score was 15. She was referred to the Psychiatry Department for further management.

Our patient had a lower quality of life at the beginning treatment of MDR-TB. Assessment HRQoL of the patient using patient-centered PedsQL showed the lowest score in physical health and emotional function. Assessment HRQoL using parent-centered PedsQL revealed the lowest scores in physical-health and school function. The assessment results of HRQoL using PedsQL, both patient and parents-centered, at six months after completing treatment revealed that the lowest score among the three functions was in physical-health function.

Discussion

The patient has been treated for pulmonary tuberculosis using appropriate regimens and durations, but she showed no improvement. In general, the possible reasons for treatment failure were non-adherence, drug resistance, drug malabsorption, laboratory errors, and patient's biological variation (polymorphism) of drugs metabolism, mutation and strain of MTB.[12-14] In our patient, drug resistance, patient's biological variation (polymorphism) of drugs metabolism, mutation and strain of MTB were couldn't be evaluated. There are two types of drug-resistant TB, primary and secondary. Primary drug-resistant TB means that the patient was infected with resistant MTB, while secondary drug-resistant TB means that the patient acquired drug resistance during TB treatment, due to spontaneous mutation in the genetic material of the mycobacteria.[15] Our patient likely had secondary drug-resistant TB, as several factors could led to spontaneous mutations. The first was social factor, including medication discontinuation and non-adherence. Patient refused to be referred after completing six months of treatment, despite experienced no clinical improvement. The second factor was the presence of fibro-cavitary lesion, which may have prevented MTB from exposure to optimum levels of the drugs.[16] The third factor was malnourished nutritional status, which cause poor drug absorbtion in gut. In addition, low serum albumin levels cause increased levels of free drugs in circulation, resulting in increasing renal clearance of drugs. Both mechanisms result in low serum drug levels and potentially subsequent drug resistance.[17]

GeneX-pert examination revealed positive. Sputum culture revealed MTB growth resistant to rifampicin and INH. Patient received MDR-TB drug treatment for children in accordance with management of adult patients.[18,19] During the treatment, many patients experience side effects and adverse drugs reaction. Second and third-line anti-tuberculous drugs often associated with side effects and adverse drug reactions, causing discontinuation and even a change in treatment regimen. Study reported that of 39 patients with MDR TB, 41% experience some side effects and 21.1% required discontinuation or change in medication.[20] Close monitoring is necessary to identify side effects early. The most common side effects are rash, gastrointestinal symptoms (nausea, vomiting, and diarrhea), psychiatric symptoms (psychosis, anxiety, depression, and suicidal thoughts), jaundice, ototoxicity, and peripheral neuropathy.[8,20,21]

Sequelae of TB on the lungs cause a decrease in lung capacity. Spirometry test in our patient revealed FEV1 predictive value of 51.1%, indicate the presence of restrictive abnormality. However, FEV1/FVC was 98.9% predictive value, means that there was no obstructive

abnormality. Mild physical activity was not impaired in this patient, but moderate was. Respiratory symptoms is generally not seen in patients with chronic lung disease until FEV1 reach 50% of predictive value.[22] Tuberculous destroyed lung (TDL) respiratory disorder caused by anatomical abnormalities, such as damage to the bronchi due to extensive fibrosis and stricture of endobronchial tissue that obstruct the airway.[23] Asymptomatic patients may not require therapy, but patients with severe disease require multiple modalities, include hospitalization for respiratory disorders.[24] Tuberculous destroyed lung with severe clinical symptoms such as massive hemoptysis, empyema, secondary fungal infection, secondary amyloidosis, septicemia, and pulmonary-systemic shunt often need surgery to improve clinical symptoms and quality of life.[25]

Long term, this lung damage can lead to heart failure due to high pressure in the pulmonary vasculature. Echocardiography examination of patient showed an increase in pulmonary blood vessels. Echocardiography revealed pressure gradient ≥40 mmHg in pulmonary artery and considered to be pulmonary hypertension.[26,27] However, the gold standard diagnostic modality of pulmonary hypertension is right heart catheterization (RHC). But a study comparing RHC and echocardiography reported that echocardiography is a reliable and valid diagnostic method for pulmonary hypertension.[28] Administration of sildenafil citrate for pulmonary hypertension in pulmonary fibrosis causes selective pulmonary vasodilation of blood vessels in the area of ventilation without interrupting gas exchange in pulmonary vasculature.[28,29]

Patient underwent physiotherapy and bracing for her scoliosis. Evaluation of six months revealed improvement in endurance, six-minute walking test increased from 1.5 to 24 minutes. She also had a clinical improvement in the scoliosis, as indicated by Cobb angle, from 53° to 50°. The basic objectives of comprehensive conservative treatment of idiopathic scoliosis are: 1) to stop curve progression at puberty (or possibly even reduce it); 2) to prevent or treat respiratory dysfunction; 3) to prevent or treat spinal pain syndromes; and 4) to improve aesthetics via postural correction.[30] Patient's bone maturation was stage 3 Risser classification, adolescent, and Cobb angle was over 50° why full-time bracing was appropriate for the management of scoliosis of the patient. Surgery is another choice if the bracing result was not sa.[31,32]

During the observation, patient experienced symptoms of mental and emotional disorder. Her initial PSC-17 evaluation revealed internalization score 8, externalization score 12, attention score 8, and total score 28 and improved to internalization score was 5, externalization score was 6, attention score was 4, and total score 15. Internalizing disorders persisted until the treatment was completed, manifest as depression and mood disorder. Depression is a mental disorder that occur in TB patients, with a prevalence of 40% to 80%.[33] There is a direct relationship between physical injury and mental health deterioration. Increasing in production of interleukin-6 mediates endocrine cascade reactions that causes depression.[34,35] Gender also has an effect on the occurrence of mental health deterioration, some studies reported mental health deterioration in TB is more common in female.[35] Biological processes, self-image, and coping mechanism in women more often lead to depression than men.[36]

The overall score of HRQoL using PedsQL was low during observation, but it increased greatly over time compared to the beginning of the observation. Chronic disease symptoms and damage due to MDR-TB, as well as the duration of therapy using drugs with toxic properties can cause many residual disorders after treatment completion.[37]

In conclusion, MDR-TB causes illnesses, physical activity limitation, physical disability, nutritional disorder, and mental health disorder. Long-term follow up with multidisciplinary approach can be expected to improve the quality of life patients with chronic disease. Monitoring medical problem and quality of life of patient should be continued to meet better outcome. Psychosocial support is necessary so they can be accepted and be involved in their environment.

Conflict of Interest

None declared.

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Original Article

Risk factors of stunting in children aged 24-59 months

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Abstract

Background Childhood stunting (low height-for-age) still remains a global health problem because it increases the risk of disturbances in growth and development as well as mortality. The prevalence of stunting in Bali is 32.5%, with the highest in Gianyar District at 41%. However, little is known about the risk factors of stunting children in Gianyar.

Objective To investigate the risk factors of stunting in children aged 24-59 months in Gianyar.

Methods This cross-sectional study involved 166 children, collected consecutively, aged 24-59 months, who visited the integrated health posts in 13 community health centers in Gianyar District, Bali, from September to November 2016. Stunting is defined as -2SD below the WHO height-for-age *z*-score (HAZ), according to sex. Statistical analyses were done with Chi-square and multivariate logistic regression tests.

Results Of 166 subjects, 37 (22.3%) children were stunted. Multivariate analysis revealed that low paternal education (AOR 2.88; 95%CI 1.10 to 7.55; P=0.031), maternal height less than 150 cm (AOR 7.64; 95%CI 2.03 to 28.74; P=0.003), high risk maternal age (AOR 4.24; 95%CI 1.56 to 11.49; P= 0.005), low birth weight (AOR 5.09; 95%CI 1.03 to 25.31; P=0.047), and low birth length (AOR 9.92; 95%CI 1.84 to 53.51; P=0.008) were strongly associated with stunting.

Conclusion Risk factors for stunting in children are low paternal education, maternal height less than 150 cm, high risk maternal age, low birth weight, and low birth length. **[Paediatr Indones. 2018;58:205-12; doi: http://dx.doi.org/10.14238/pi58.5.2018.205-12]**.

Keywords: *stunting; children; risk factor*

Childhood stunting (low height-for-age) is one of the most significant health problems that should not be ignored in the public health realm. This kind of chronic malnutrition restricts a child's potential growth due to inadequate nutritional intake. Stunting, or being too short for one's age, is defined as below 2 standard deviations (SD) from the median height-for-age *z*-score (HAZ), as determined by the *World Health Organization* (WHO) Child Growth Standards.[1,2]

Globally, between 171 to 314 million children are stunted, located predominantly in African and Asian regions.[3,4] In 2010, 26.7% of children in Asia and also 26.7% in South East Asia were stunted.[5] According to the *Indonesian Basic National Health Survey* (*Riset Kesehatan Dasar*), the prevalence of stunting in Indonesia was 36.8% in 2007, decreased to 35.6% in 2010, and increased to 37.2% in 2013.[6-8]

Infants are a special population with a critical growth and development period.[9] Inadequate nutrition in infancy leads to problems later in life.

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Nutritional problems such as stunting can also be harmful to infants because it can lead to emotional, social and cognitive development problems in adulthood. In addition, childhood stunting increases the risk of mortality, deficits in cognitive function, poor motor development, and loss of physical growth potential.[10,11] The long-term consequences of stunting in children can lead to disproportions of body stucture, unfulfilled academic potential, poor reproductive health, and increased risk of infection.[12]

Studies on stunting risk factors in developing countries have yielded diverse results and site differences. Thus, the risk factors for stunting remain inconclusive.[13] A previous study reported that severe household food insecurity and lower socio-economic status were significant contributors to stunting incidence in Southeastern Kenya.[14] In addition, another study in Nepal showed that low family income and prolonged breastfeeding for more than 12 months were significant risk factors for stunting.[15] A study in India showed that maternal education, age, and body mass index (BMI) were associated with stunting.[4] To our knowledge, there is lack of national data concerning stunting determinants in Indonesia.

The prevalence of stunting in Bali is 32.5%, with the highest prevalence in the Gianyar District (41%).[16] This figure was higher compared to both national and global reports, thus, stunting remains an important issue to be tackled.[1,8] There is a lack of data regarding the risk factors for stunting in Balinese children, particularly in the Gianyar District. Therefore, to better understand stunting and the risk factors associated with stunting, we conducted this study among children aged 24-59 months in the Gianyar District of Bali.

Methods

This cross-sectional, analytic study was conducted from September to November 2016. We consecutively included 166 children aged 24-59 months who had attended the growth monitoring program in integrated health posts (Posyandu) at thirteen community health centers (Puskesmas) in the Gianyar District, Bali, during the study period. Children who resided in the district, lived with their parents, and had maternal and child health books (*Buku Kesehatan Ibu dan Anak*/KIA), as well as health record cards (*Kartu Menuju Sehat*/KMS) published by the Ministry of Health, Republic of Indonesia were included in the study. However, those with mental disorders, disabilities, or whose mothers refused consent were excluded.

Subjects' data were obtained from anthropometry measurements and interviews using questionnaires. Primary data were obtained to determine socio-demographic items consisting of age, sex, highest level of education, monthly family income, and parity. The secondary data were obtained from the KIA book and KMS record to elicit maternal health, birth information, and the development of infant growth from birth to 5 years of age. Also included were maternal height, mid-upper arm circumference, gestational age, birth weight and length, exclusive breastfeeding status, and immunization records. Children's heights were measured with head facing forward and standing in an upright position without footwear, using a *One Med®* microtoise with 1 mm accuracy.

The dependent variable was the classification of stunting and non-stunting. Stunting was defined as WHO HAZ below -2 SD, according to sex.[1,8] Independent variables for parents were education level, family income, maternal height, mid-upper arm circumference, maternal age, parity, and gestational age; variables for children were sex, age, birth order, birth weight, birth length, exclusive breastfeeding, and immunization status. Parental education level was defined as low if the highest education completed was below high school. Family income category was based on the monthly minimum wage of Gianyar (*Upah Minimum Kabupaten*/UMK), which was IDR 1,904,141 in 2016.17 Maternal height was categorized as short if <150 cm.[8] Mid-upper arm circumference was small if ≤ 23.5 cm.[8] High risk maternal age at pregnancy was defined as less than 20 or more than 35 years.[8] Gestational age was categorized as preterm for 37 weeks, and parity was categorized as less than or equal to 2.[8] Birth order of children was categorized as first or not first. Low birth weight was defined as birth weight $<2,500$ grams, and low birth length as <48 cm.[8] The children were considered to not have received exclusive breastfeeding if stopped before 6 months of age. Immunization status was incomplete if the children had missed one or more of the following: BCG, DPT, polio, measles, and hepatits B vaccine.[8]

The questionnaires, secondary data from samples, and anthropometric data were then analyzed using *SPSS version 20* software. Descriptive analysis was used to show the frequency of variables and mean differences. Chi-square bivariate test and odds ratio (OR) were performed to assess the relationship between possible risk factors and stunting, whereas multivariate analysis by logistic regression was used to calculate the adjusted odds ratio (AOR) for defined variables that were significant by Chi-square analysis. The results were considered statistically significant for P<0.05 with 95% confidence interval (95%CI). The study was approved by the Ethics Committee of Universitas Udayana Medical School/Sanglah Hospital, Denpasar, Bali.

Results

This study involved a total of 166 children aged 24-59 months who had visited one of thirteen community health centers in Gianyar District, Bali. The majority of subjects were male (54.2%) and in the age range of 24-35 months (41.6%). Thirty-seven (22.3%) children were stunted, based on their HAZ. There was no significant difference in ages of stunted vs. non-stunted subjects (P=0.64). The baseline characteristics of subjects and parents are shown in **Table 1**.

Table 1. Sociodemographic characteristics of parents and children

Characteristics	Total (N=166)	P value
Parents		
Paternal education, n (%)		
Low	45 (27.1)	
High	121 (72.9)	
Maternal education, n (%)		
Low	61 (36.7)	
High	105 (63.3)	
Family income, n (%)		
< *UMK*	41 (24.7)	
≥ *UMK*	125 (75.3)	
Maternal height, n (%)		
Short (<150 cm)	13 (7.8)	
Normal (≥150 cm)	153 (92.2)	
Mid-upper arm circumference, n (%)		
Small (≤ 23.5 cm)	8 (4.8)	
Normal (> 23 cm)	158 (95.2)	
Maternal age, n (%)		
High risk (< 20 or > 35 years)	30 (18.1)	
Ideal (20 – 35 years)	136 (81.9)	
Parity, n (%)		
≤ 2	139 (83.7)	
> 2	27 (16.3)	
Gestational age, n (%)		
Preterm (< 37 weeks)	16 (9.6)	
Full term (≥ 37 weeks)	150 (90.4)	
Children		
Sex, n (%)		
Male	90 (54.2)	
Female	76 (44.8)	
Age, n (%)		
24-35 months	69 (41.6)	
36-47 months	53 (31.9)	
48-59 months	44 (26.5)	
Birth order, n (%)		
First	76 (45.8)	
Not first	90 (54.2)	
Birth weight, n (%)		
Low (< 2,500 grams)	11 (6.6)	
Normal (≥ 2,500 grams)	155 (93.4)	
Birth length, n (%)		
Low (< 48 cm)	7 (4.2)	
Normal (≥ 48 cm)	159 (95.8)	
Exclusive breastfeeding, n (%)		
No	8 (4.8)	
Yes	158 (95.2)	
Immunization status, n (%)		
Incomplete	9 (5.4)	
Complete	157 (94.6)	
Height-for-age, n (%)		
Stunted (< -2 SD)	37 (22.3)	
Non-stunted (≥ -2 SD)	129 (77.7)	
Mean age (SD), months		
Stunted	39.84 (10.20)	0.64
Non-stunted	38.91 (10.51)	
Mean height (SD), cm		
Stunted	87.71 (8.78)	<0.001*
Non-stunted	95.80 (7.57)	

UMK (*Upah Minimum Kabupaten*)=district minimum wages ; *statistically significant

Bivariate analysis was used to compare nutritional status (stunted and non-stunted) and the independent variables (possible risk factors). It revealed that low paternal education (OR 2.63; 95%CI 1.22 to 5.68; P=0.012), low maternal education (OR 2.53; 95%CI 2.12 to 5.32; P=0.013), short maternal height (OR 4.78; 95%CI 1.5 to 15.28; P=0.04), high risk maternal age (OR 4.3; 95%CI 1.85 to 10; P < 0.001), low birth

weight (OR 7.29; 95%CI 2 to 26.53; P= 0.001), low birth length (OR 9.92; 95%CI 1.84 to 53.5; P=0.01), and didn't receive exclusive breastfeeding (OR 6.56; 95%CI 1.49 to 28.9; P=0.05) were statistically significant. However, variables such as small mid-upper arm crcumference, parity ≤ 2, preterm birth, child sex, birth order, and incomplete immunization status were not associated with stunting (**Table 2**). The following variables were significant or had P values < 0.25, and were further analyzed using the logistic regression model: low paternal education (P=0.012), low maternal education (P=0.013), short

Table 2. Possible risk factors of stunting based on bivariate analysis

Variables	Nutritional status		OR	95%CI	P value
	Stunted (n=37)	Non-stunted (n=129)			
Paternal education, n (%)					
Low	16 (35.6)	29 (64.4)	2.63	1.22 to 5.68	0.012*†
High	21 (17.4)	100 (82.6)			
Maternal education, n (%)					
Low	20 (32.8)	41 (67.2)	2.53	2.12 to 5.32	0.013*†
High	17 (16.2)	88 (83.8)			
Family income, n (%)					
< UMK	12 (29.3)	29 (70.7)	1.66	0.74 to 3.70	0.216†
≥ UMK	25 (20)	100 (80)			
Maternal height, n (%)					
Short (<150 cm)	7 (53.8)	6 (46.2)	4.78	1.50 to 15.28	0.004*†
Normal (≥150 cm)	30 (19.6)	123 (80.4)			
Mid-upper arm circumference, n (%)					
Small (≤ 23.5 cm)	1 (12.5)	7 (87.5)	0.48	0.06 to 4	0.495
Normal (> 23 cm)	36 (22.8)	122 (77.7)			
Maternal age, n (%)					
High Risk (< 20 and > 35 years old)	14 (46.7)	16 (53.3)	4.3	1.85 to 10	< 0.001*†
Ideal (20-35 years old)	23 (16.9)	113 (83.1)			
Parity, n (%)					
≤ 2	33 (23.7)	106 (76.3)	1.79	0.58 to 5.55	0.308
> 2	4 (14.8)	23 (85.2)			
Gestational age, n (%)					
Preterm (< 37 weeks)	5 (31.2)	11 (68.8)	1.67	0.54 to 5.17	0.365
Full term (≥ 37 weeks)	32 (21.3)	118 (78.7)			
Child sex, n (%)					
Male	16 (21.1)	60 (78.9)	0.88	0.42 to 1.83	0.725
Female	21 (23.3)	69 (76.7)			
Birth order, n (%)					
First	17 (22.4)	59 (77.6)	1	0.48 to 2,10	0.982
Not first	20 (22.2)	70 (77.8)			
Birth weight, n (%)					
Low (< 2,500 grams)	7 (63.6)	4 (36.4)	7.29	2 to 26.53	0.001*†
Normal (≥ 2,500 grams)	30 (19.4)	125 (80.6)			
Birth length, n (%)					
Low (< 48 cm)	5 (71.4)	2 (28.6)	9.92	1.84 to 53.5	0.001*†
Normal (≥ 48 cm)	32 (20.1)	127 (79.9)			
Exclusive breastfeeding, n (%)					
No	5 (62.5)	3 (37.5)	6.56	1.49 to 28.92	0.005*†
Yes	32 (20.3)	126 (79.7)			
Immunization status, n (%)					
Incomplete	3 (33.3)	6 (66.7)	1.8	0.43 to 7.60	0.413
Complete	34 (21.7)	123 (78.3)			

*Statistically significant; P value is based on bivariate analysis using Chi-square test
†Possible risk factors with P<0.25 were included in multivariate analysis model using logistic regression

maternal height (P=0.04), high risk maternal age (P < 0.001), low birth weight (P=0.001), low birth length (P=0.01), not exclusively breastfed (P=0.05), and family income < UMK (P=0.216).

Multivariate analysis revealed that low paternal education (AOR 2.88; 95%CI 1.10 to 7.55; P=0.031), short maternal height < 150 cm (AOR 7.64; 95%CI 2.03 to 28.74; P=0.003), high risk maternal age at pregnancy (AOR 4.24; 95%CI 1.56 to 11.49; P=0.005), low birth weight (AOR 5.09; 95%CI 1.03 to 25.31; P=0.047), and low birth length (AOR 9.92; 95%CI 1.84 to 53.51; P=0.008) were strongly associated with stunting (**Table 3**).

Table 3. Multivariate analysis of risk factors of stunting

Variables	Adjusted OR	95% CI	P value
Paternal education	2.884	1.10 to 7.55	0.031**
Maternal education	1.241	0.47 to 3.25	0.660
Maternal height	7.640	2.03 to 28.74	0.003**
Maternal age	4.239	1.56 to 11.49	0.005**
Birth weight	5.092	1.03 to 25.31	0.047**
Birth length	9.92	1.84 to 53.51	0.008**
Ecxlusive breastfeeding	2.795	0.40 to 19.66	0.302

** Statistically significant; P value is based on multivariate analysis model using logistic regression

Discussion

Childhood stunting is a type of malnutrition with potentially irreversible outcomes due to poor nutritional intake. It often goes unrecognized in communities. Stunting has long-term effects beyond the individuals, as societies are affected by by populations of people with lack of cognitive skill, delayed physical development, and risk of chronic diseases.[1] We found stunting in 37 out of 166 subjects (22.3%). This finding was quite different compared to the data based on Riskesdas in 2013 (41%). The factor that might contributted in declining the proportion of stunting was the intervention performed by either local government or Puskesmas, and Posyandu after the high prevalence finding, such as Local Action Planning (*Recana Aksi Daerah*), education and training for health workers in puskesmas and posyandu towards the community. Gianyar District is also included in 100 priority areas in Indonesia to combat stunting.[18]

Our results on the correlation of low paternal education to stunting in children are supported by other studies. Two studies in West and Central Java reported that paternal education was a risk factor of poor nutritional status in children, leading to stunting.[19,20] A Bogor study found that parents with higher education may have better understanding of children's nutritional requirements, growth, and development, which may lead to provide better care of their children.[21] This finding was also supported by the *World Health Organization (WHO) Conceptual Framework on Childhood Stunting* that noted poor care practices and low caregiver education as causes of stunting in children.[22]

Short maternal stature was also a risk factor for stunting prevalence noted by the WHO,[22] similar to our study. Several studies in Indonesia also reported that maternal height contributed to stunting prevalence.[20,23,24] Studies revealed that short maternal stature was associated with growth failure in children, and short mothers tended to have stunted children at 2 years.[25] The interaction between maternal stature and linear growth of children is likely due to genetics and environmental aspects overseen by mothers, such as hygiene, adequate nutritional intake, and reproductive health.[25,26] Mothers with short stature might have inadequate anatomical and metabolic systems which may affect maternal and fetal health, such as lower glucose level, or decreased protein and energy. These conditions may lead to intrauterine growth restriction, which also plays a role in short stature in children.[25,27,28]

Child nutritional status also can be influenced by maternal age. Young maternal age at childbearing was associated with increased risk of preterm birth, intrauterine growth restriction, mortality of both infant and mother, and undernutrition.[29] Young mothers also generally had lower nutritional status than older mothers, thus manifesting as low pre-pregnancy weight (under 50 kg) and/or weight gain during pregnancy of less than 10 kg.[30] Less than ideal maternal nutritional status can increase the risk in having a low birth weight child, which makes them prone to stunting.[31] On the other hand, older mothers also have higher pregnancy risk, increased risk of stillbirth, preterm birth, intrauterine growth restriction, and chromosomal abnormality.[29] We found a significant association between stunting and high risk maternal age (<20 years or >35 years old).

Our study found that low birth weight and length had significant associations with stunting. Indonesian studies by Kuntari,[32] Oktarina & Sudiarti,[24] and Rahayu[33] also showed a significant association between low birth weight and stunting. Birth weight is an important predictor of child body size in the next phase of their growth and development.[34] Children with low birth weight (under 2,500 grams) have higher risk of malnutrition, infection, and degenerative diseases. Malnutrition and infection may negatively affect growth and development and increase child morbidity later in life.[35] Rahayu *et al.* found that low birth length was significantly associated with stunting prevalence in children aged 6-12 months. However in children aged 36-48 months, low birth length was not significantly associated with stunting prevalence. This condition may be because low birth length may have a greater effect at early ages, but be overcome to result in normal growth later on.[33] Another study by Utami *et al.* in Bogor showed that low birth length had a significant association with children aged 0-23 months. This circumstance may be due to genetic factors or poor maternal nutritional status during pregnancy.[36]

Breast milk is recognized as essential feeding for infants for the first six months of life. The *WHO* and the *Indonesian Ministry of Health* recommend exclusive breastfeeding, because it provides adequate nutrition and has advantages over formula, such as in developing brain function, increased immune system function, and enhancing infant growth and development.[37] Our study showed that lack of exclusive breastfeeding had an association with stunting, based on bivariate analysis. The WHO framework of child stunting stated that inadequate breastfeeding, such as non-exclusive breastfeeding, delayed initiation, or early cessation of breastfeeding are significantly associated with stunting cases.[38] Indonesian studies also showed that non-exclusive breastfeeding increased the risk of stunting.[33,39] However, in our study, lack of exclusive breastfeeding was not a significant risk factor for stunting by multivariate analysis.

The limitations of this study were the cross-sectional design which does not reveal causal relationships between the variables. In addition, several variables that might be significant risk factors for stunting in children were not investigated in this study, such as infectious disease (diarrhea, helminth infection, or upper respiratory tract infection), sanitation of the house and environment, supplemental feeding, and parental knowledge on child nutrition. Thus, we suggest future studies with better methodologies, larger sample size, and more variables. Further studies are needed to combat and eradicate stunting by intervening to reduce the risk factors.

In conclusion, the proportion of stunted children aged 24-59 months in Gianyar District is 22.3%. Low paternal education, short maternal height, high risk maternal age, low birth weight, and low birth length are significantly associated with stunting.

Conflict of interest

None declared.

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Original Article

Forced expiratory volume in 1-second and blood gas analysis in children during asthma attacks

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Abstract

Background Asthma is the most common chronic disease in the world, with a high incidence in children. Blood gas analysis and pulmonary function test using spirometry are recommended to evaluate the degree of asthma in children. Spirometry test is non-invasive and easier to implement compared to blood gas analysis.

Objective To evaluate for a possible correlation between forced expiratory volume in 1 second (FEV1) measured by spirometry test and blood gas analysis (pO_2 and pCO_2 levels) in children during an asthma attack.

Methods This cross-sectional study was done in children with asthma attacks who were admitted to Sanglah Hospital, Denpasar, Bali, between November 2016 and April 2017. Subjects underwent spirometry tests and blood gas analyses. Potential correlations between FEV1 and pO_2 and pCO_2 levels were analyzed by Spearman's correlation test.

Results A total of 50 subjects, consisting of children aged 6 to 12 years, were diagnosed with asthma attacks during the study period. Subjects' mean FEV1 level was 43.6%, mean pCO_2 was 38.36 mmHg, and mean pO_2 was 121.92 mmHg. There were no significant correlations between FEV1 and pCO_2 level (r=0.206; P=0.152) or FEV1 and pO2 (r=0.157; P=0.277) found in this study.

Conclusion FEV1 does not correlate with pCO_2 and pO_2 level in children during asthma attacks. **[Paediatr Indones. 2018;58:221-6; doi: http://dx.doi.org/10.14238/pi58.5.2018.**221-6 }

Keywords: *spirometry; blood gas analysis; asthma; children*

Asthma remains a serious problem worldwide, since it is the most common chronic disease in children and adults.[1] Approximately 300 million people around the world have been diagnosed with asthma. The asthma prevalence in children aged 5-14 years in the US reached 69.8 cases per 1,000 children.[2] The prevalence in Indonesian children is unknown, but in adults approximately 10% of 25 million Indonesians have asthma with high morbidity and mortality.[3]

An asthma attack is an emergency requiring oxygenation, ventilation, and acid-base management.[4] Optimal management includes not only symptom control, but lung function monitoring and blood gas analysis.[1] Lung function test is necessary to assess severity, obstruction, reversibility, and diagnostic accuracy of the asthma. Spirometry is recommended at least once a year in children with asthma to assess respiratory function.[5] Decreased FEV1 can be used to assess the degree of obstruction. Variation in FEV1 is also a good predictor of asthma severity.[6]

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Blood gas analysis is recommended for all asthma attack patients who come to the hospital. Blood gas analysis results are a good estimate of asthma severity. More severe obstruction tends to correlate with higher CO_2 and lower pH in arterial blood.[7] Blood gas analysis is more invasive and traumatic for children compared to spirometry. Several studies were done to assess for a correlation of FEV1 decrease with pO_2 and pCO_2 level in adults with obstructive respiratory diseases, but with varying results.[8,9] We evaluated for correlations between FEV1 decrease and pO_2 and pCO_2 levels in children with asthma attacks.

Methods

This cross-sectional study was performed in the Emergency Department of Sanglah Hospital, Denpasar, Bali, from November 2016 - April 2017. Subjects were children diagnosed with asthma, aged >6 years, and brought to the Emergency Department due to asthma attacks. Study subjects were recruited using consecutive sampling until the minimum required sample size was achieved. The sample size was determined for a cross-sectional study with 5% significance level (α) and 80% power (β), and estimated to be 50 from minimal difference in previous studies.[10,11]

Subjects classified to mild-moderate and severe asthma attack based on clinical finding. The clinical findings of mild-moderate asthma attacks were shortness of breath, no exertion of additional respiratory muscle, spoke in sentence, prefer in sitting position, and a loud expiratory-inspiratory wheeze on auscultation. While in severe asthma attacks, the clinical findings were shortness of breath, exertion of additional respiratory muscles, difficulty speaking, leaning forward sitting position, irritable, and a loud expiratory-inspiratory wheeze can be heard without a stethoscope.[12,13]

Exclusion criteria were children diagnosed with impending respiratory failure, chronic lung disease, acute or chronic lung infection, congenital lung diseases, heart diseases, history of lung surgery, or systemic diseases that impaired lung function. Subjects' parents provided written informed consent. This study was approved by the Human Study Ethics Committee of Sanglah Hospital.

Subjects underwent history-taking and physical examinations. Spirometry and blood gas analysis were performed after assessment before bronchodilator therapy. Blood specimens were collected in containers with anti-coagulant (heparin) for blood gas analyses using *Siemens RapidLab 348Ex®*. Diagnoses of asthma and degree of severity were made based on *National Pediatric Asthma Guidelines (Pedoman Nasional Asma Anak Indonesia)*.[12]

Characteristics of subjects were described in tables. Differences in FEV1, pO2, and pCO2 were analyzed using independent T-test or Mann-Whitney test, depending on data normality. Spearman's test was performed to analyze abnormal data distributions. Analyses were performed with *SPSS 22.0* software.

Results

A total of 50 subjects were included in this study between November 2016 and April 2017. There were 10 children with severe asthma attacks and 40 with mild-moderate asthma attacks. The male: female ratio was 2.3: 1. Characteristics of subjects are shown in **Table 1**.

Table 1. Characteristics of subjects

Characteristics	(N = 50)
Mean age (SD), years	9.06 (2.123)
Sex, n (%)	
Male	35 (70)
Female	15 (30)
Asthma severity, n (%)	
Severe	10 (20)
Mild–moderate	40 (80)
Mean FEV1 (SD), %	43.60 (16.54)
Mean PCO_2 (SD), mmHg	38.36 (8.89)
Mean PO_2 (SD), mmHg	121.92 (42.35)

Kolmogorov-Smirnov test revealed that FEV1 data were normally distributed, but pO_2 and pCO_2 data were not normally distributed. We found that FEV1 had no significant correlations with pO_2 or pCO_2, as shown in **Table 2**.

Regression correlation test on FEV1 with pO_2 and pCO_2, based on asthma severity, revealed differences in severe attack compared to mild-moderate attack.

A stronger correlation was found in severe asthma attack compared to mild-moderate attack, as seen on the scatter plots in **Figure 1**.

Table 2. Correlation of FEV 1 with pO_2 and pCO_2

		pCO_2	pO_2
FEV1	r	-0.206	0.157
	P*	0.152	0.277

*Spearman correlation test

Further analyses of FEV1, pCO_2, and pO_2 based on asthma severity were performed. Parametric analysis was performed on FEV1 and pCO_2, and non-parametric analysis was performed on FEV1 and pO_2, due to differences in data normality. Significant mean differences of FEV1, pO_2, and pCO_2 were observed according to asthma severity, as shown in **Table 3**. Children with severe asthma attacks had a significant lower FEV-1 and pO_2, and significant higher pCO_2 compared to children with mild to moderate asthma attacks.

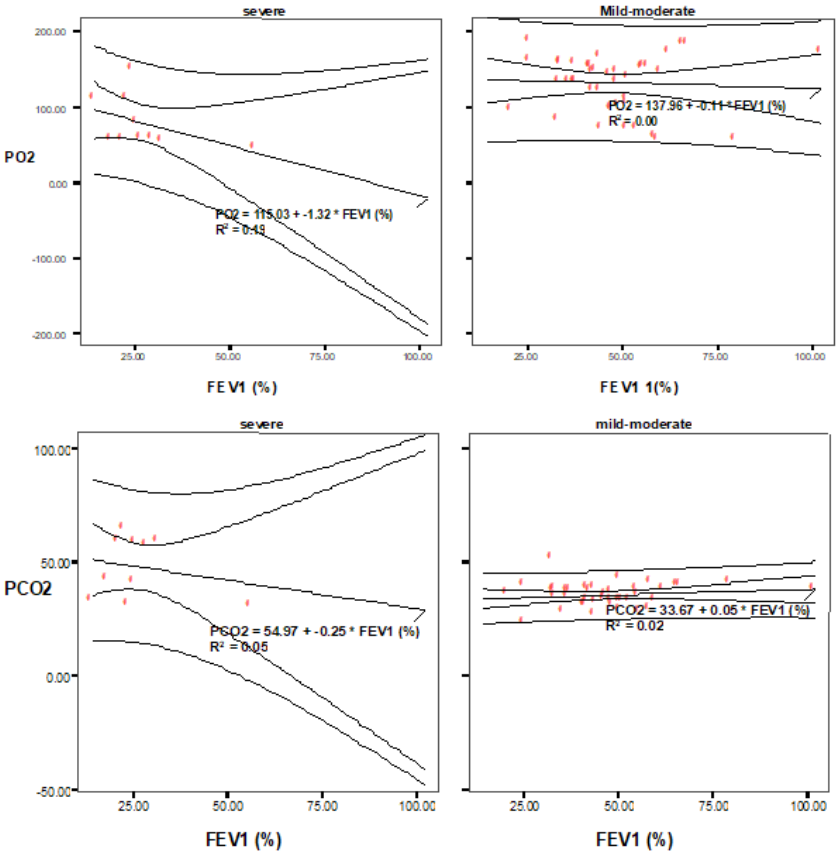

Figure 1. Correlation of FEV1 with pCO_2 and pO_2, based on asthma severity

Table 3. Differences of FEV1, pO_2, and pCO_2 levels, based on asthma severity

Variables	Asthma severity		P value
	Severe	Mild-moderate	
Mean FEV1 (SD), %	26.99 (11.5)	47.75 (15.0)	0.002[a]
Mean PCO2 (SD), mmHg	48.20 (13.40)	35.90 (5.17)	0.018[b]
Mean PO2 (SD), mmHg	79.39 (34.81)	132.55 (37.34)	0.001[a]

a: Mann-Whitney test; b: Independent T-test

Discussion

Asthma is defined as chronic inflammation of the airway. Many cells types and cellular elements have roles in its pathogenesis. This chronic inflammation is related to bronchoconstriction, airway swelling, airway hyperresponsiveness, and remodelling.[1,13,14] The natural history of the disease usually starts in childhood, and continues to impose a high economic burden, high morbidity and mortality, as well as reduced quality of life.[1,7]

Diagnosis of asthma in children is based on episodic and reversible airway obstruction or airway hyperresponsiveness, when other differential causes have been excluded. Diagnosis can be made by history-taking, physical findings, and spirometry test. Spirometry can be used to assess the degree of obstruction and reversibility in children over 5 years of age. This test is difficult to perform in younger children. Other examinations are used to exclude other causes.[15,16] Blood gas analysis is the gold standard examination for assessing gas exchange, arterial oxygen status, and acid-base status.[4,17]

The Global Initiative for Asthma (GINA) has recommended spirometry test and blood gas analysis to assess the severity of asthma attacks.[1] Spirometry is valuable for assessing airway patency and degree of obstruction, while blood gas analysis is valuable to assess gas exchange and ventilation/perfusion. The FEV1 levels vary according to the severity of the attack: mild >60%, moderate 40-60%, and severe <40%. Blood gas analysis cut-off points for severity are as follows: mild has normal pO_2, pCO_2 <45 mmHg, and SaO_2 >95%; moderate has pO_2 >60 mmHg, pCO_2 <45 mmHg, and SaO_2 91-95%, and severe has pO_2 <60 mmHg, pCO_2>45 mmHg, and SaO_2 ≤90%.[12]

The mean age of our subjects was 9 years, similar to other studies that showed most asthma attacks occurred in children aged 6-12 years.[12] A Bandung study in 2012 found an asthma prevalence of 9.6% in children aged 7-14 years. We had more male subjects than females, with a male: female ratio of 2.3:1. Another study also noted more males with asthma attacks.[7] The mean FEV1 level in our subjects was 43.6%, which was in the mild-moderate asthma attack range. Subjects' mean pCO_2 level was 38.36 mmHg and mean pO_2 was 121.92 mmHg, which were also consistent with mild-moderate attack severity, as 80% of our subjects had mild-moderate attacks.

The aim of the study was to assess for a possible correlation between blood gas analysis and spirometry results. Spirometry is a non-invasive examination and easy to perform in children, while blood gas analysis is invasive and difficult to perform. We had hoped that spirometry results could be used to predict blood gas levels, however, we found no significant correlation between FEV1 and pCO_2 level (r=-0.206; P=0.152) nor between FEV1 and pO_2 level (r=0.157; P=0.277). To our knowledge, such a study has not been done in children. A chronic obstructive pulmonary disease (COPD) study in adult subjects in 2004 showed a significant weak correlation between FEV-1 with pCO_2 and pO_2.[8] This difference might be due to childhood asthma being a reversible disease, unlike the chronic, persistent COPD, in which only severe attacks change pO_2 and pCO_2 levels. Different results in adult subjects may also be due to greater cooperativity during spirometry, compared to our pediatric subjects.

Further data analysis revealed significant differences between asthma severity groups in terms of FEV1, pO_2, and pCO_2 levels. Mean FEV1 was significantly lower in the severe asthma attack group compared to the mild-moderate attack group (26.99 *vs.* 47.75%, respectively; P=0.002), similar to another study.[18] Lower FEV1 was also correlated to airway reversibility.10 In addition, mean pCO_2 level was significantly higher in the severe group than in the mild-moderate group (48.2 *vs.* 35.9 mmHg, respectively; P=0.018). Padmavathi *et al.* found hypercapnia in 45% of patients with severe attacks.[11] The pCO_2 levels are considered to be 41-60 mmHg in severe attack and <40mmHg in mild-moderate attack.[14] In our study, mean pO_2 level was 132.55 mmHg in the mild-moderate attack group and 79.39 mmHg in the severe attack group (P=0.001). This result was similar to another study that showed hypoxemia in 55% of cases of severe asthma attack.[11]

Blood gas analysis has low specificity and cannot be used to assess the degree of broncho constriction, hence, blood gas analysis is not suitable as a screening test for early lung disease. During asthma attacks, pO_2 gradually decreases and pCO_2 also gradually decreases due to the hyperventilation mechanism.

Levels of pO_2 and pCO_2 continue to decrease in accordance with the severity of the attack, until at some point, the inability of the lungs to dispel CO_2 leads to arterial CO_2 entrapment. This condition is only found in severe asthma attacks, while in mild-moderate attacks, increased pCO_2 and decreased pO_2 are not observed.[7] Increased pCO_2 levels can be seen if the FEV1 reaches 20-25%. As such, the lack of significant correlations in our study may have been due to our having mostly subjects with mild-moderate asthma attacks (80%). Their pO_2 and pCO_2 levels may have been less affected.

Limitations of this study were that most subjects had mild-moderate attacks, and a time lag between blood gas analysis and spirometry (spirometry was performed first while waiting for phlebotomist). Also, the child's level of cooperation might influence spirometry results.

In conclusion, there is no significant correlation between decreased FEV1 and decreased pO_2, nor between decreased FEV1 and increased pCO_2 level. The FEV1 level is significantly lower in the severe asthma attack compared to the mild-moderate asthma attack groups. Also, the level of pO_2 is significantly lower and the level of pCO_2 is significantly higher, in the severe asthma attack group compared to mild-moderate asthma attack group. Further study with a larger sample size, case-control design, and examinations performed without a time lag may yield a better understanding about asthma.

Conflict of interest

None declared.

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Original Article

NT-proBNP level and left ventricle diameters before and after transcatheter closure of PDA and VSD

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Abstract

Background Amino-terminal pro-B-type natriuretic peptide (NT-proBNP) levels before and after transcatheter closure may correlate with changes in left ventricular internal diameter end diastole (LVIDd) and end systole (LVIDs). Patent ductus arteriosus (PDA) and ventricular septal defect (VSD) are structural abnormalities which effects cardiac hypertrophy. Cardiac muscle stretching decreases after closure, followed by reduced left ventricle diameters and decreased NT-proBNP levels.

Objective To analyze for possible correlations between NT-proBNP levels and left ventricle diameters before and after transcatheter closure.

Methods Subjects were PDA and VSD patients who underwent transcatheter closure in the Pediatrics Department of dr. Moh Hoesin Hospital, Palembang, South Sumatera, from May 2016 to March 2017. Measurement of NT-proBNP levels and echocardiography were performed before closure, as well as one and three months after closure.

Results There were 34 subjects (15 girls) with median age of 91.5 months. Median NT-proBNP levels were significantly reduced after closure: before closure 111.7pg/mL, one month after closure 62pg/mL, and three months after closure 39 pg/mL ($P<0.05$). Median LVIDd and LVIDs were also significantly reduced after closure [LVIDd: 39.5mm before, 34.5mm one mo after, and 32.5mm 3 mo after ($P<0.05$); LVIDs: 23.9mm before, 20.5mm 1 mo after, and 20.0mm 3 mo after ($P<0.05$)]. At one month after closure, there was a moderate positive correlation between NT-proBNP levels and LVIDd ($r=0.432$; $P=0.011$), but no correlation with LVIDs ($r=0.287$; $P=0.100$). At three months after closure, there was a significant moderate positive correlation between changes of NT-proBNP levels and changes of LVIDd ($r=0.459$; $P=0.006$), as well as LVIDs ($r=0.563$; $P=0.001$).

Conclusion In pediatric PDA and VSD patients, NT-proBNP levels have a significant positive correlation with diastolic and systolic left ventricle diameters at three months after closure. Decreased NT-proBNP levels may be considered as a marker of closure effectiveness. **[Paediatr Indones. 2018;58:205-12; doi: http://dx.doi.org/10.14238/pi58.5.2018.205-12]**.

Keywords: *left ventricular internal diameter end diastole; left ventricular internal diameter end systole; NT-proBNP; PDA closure; VSD closure*

Amino-terminal pro-B-type natriuretic peptide (NT-proBNP) is the primary heart hormone secreted by myocytes in the right and left ventricles. This biomarker plays an important role in diagnosis, monitoring, management, and prognosis of heart disease. The NT-proBNP production is a result of fission of greater natriuretic molecules. It has several biological effects such as diuresis, vasodilatation, inhibition of renin and aldosterone production, as well as inhibition of myocyte growth in the heart and blood vessels.[1-4] The NT-proBNP levels elevate in response to increased

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volume load and excessive pressure on the heart muscle that causes left ventricular hypertrophy, with divergent patterns. Excessive pressure usually leads to concentric hypertrophy, whereas increased volume load causes eccentric hypertrophy. Heart muscle stress, such as ventricular stretching or increased filling pressure, stimulates NT-proBNP synthesis and secretion. This biomarker is directly proportional to the degree of ventricular stretching, with greater ventricular stretch causing higher NT-proBNP levels. The NT-proBNP levels are also affected by conditions such as anemia, bronchopneumonia, sepsis, heart failure, kidney failure, puberty, and increased intracranial pressure.[1-5]

Ventricular septal defect (VSD) and persistent ductus arteriosus (PDA) are structural abnormalities of the heart that can cause alteration in the heart muscle. PDA and VSD are different structural abnormalities, but with similar effects of left atrial and left ventricle hypertrophy, caused by an excessive volume load increase. The size of the defect affects the stretching of the heart muscle that stimulates NT-proBNP production by myocyte in the heart muscle. Transcatheter closure is a therapy using a special device to close the VSD and/or the PDA to reduce left ventricular volume load, thus reducing left ventricular muscle stretching followed by decreasing NT-pro BNP levels.[6-7]

In general, NT-proBNP levels decrease if the strain to the heart muscle is reduced, due to decreasing afterload and preload. Left ventricular muscle strain can be assessed through echocardiography by measuring systolic and diastolic left ventricular diameter, as well as left ventricular posterior wall thickness, which some studies found to be proportional to NT-proBNP hormone levels.[8-12]

The NT-proBNP assessment has become routine practice for the diagnosis of heart disease, as this hormone levels correlated with clinical signs of heart failure.[13] In addition, a study found a positive correlation between NT-proBNP levels and left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), and size of defects in children with VSD.10 Studies of NT-proBNP levels associated with current cardiac prognosis are still rare, especially with regard to catheterization. But one such study which assessed the effectiveness of percutaneous closure on left ventricular function by measuring NT-proBNP levels and left ventricular dimension, found that median diastolic volume and NT-proBNP levels decreased 6 months after closure.[11]

Examination of hormone levels is a tool used to assess the effectiveness of transcatheter closure in patients with congenital heart disease.[2] To assess the response of the treatment, hormone levels should be checked serially. The benefits of NT-proBNP examination are enormous, but in Indonesia, the research on NT-proBNP levels after transcatheter closure has been limited, especially in pediatrics, and, to our knowledge, has never been done at our institution.

Methods

This time series study with a correlation test design was done to assess for possible patterns and correlations between NT-proBNP levels and left ventricular diameters before and after transcatheter closure. The study subjects were children with PDA or VSD disease who underwent transcatheter closure between May 2016 and August 2017 at Dr. Mohammad Hoesin Hospital, Palembang, South Sumatera.

All subjects who met the criteria underwent echocardiographic examinations for diastolic and systolic left ventricular diameters as well as NT-proBNP measurements, before, one month, and three months after transcatheter closure. We analyzed the correlation coefficient in left ventricle diameter and NT-proBNP levels between before closure,1 month and 3 months after transcatheter closure, with Spearman's test.

Descriptive data were presented as median with range; normality test (Shapiro-Wilk) was performed on the data. Wilcoxon signed-rank test was used to compare changes of NT-proBNP levels and left ventricle diameters before and after closure. Box-and-whisker plots were used to illustrate the distribution of NT-proBNP levels. We analyzed all data with *SPSS 18* software. The Health Research Review Committee of Mohammad Hoesin Hospital approved the study protocol.

Results

Of 34 subjects who met the criteria, boys out numbered girls, and PDA patients outnumbered VSD patients

(24 *vs.* 10 subjects, respectively). The characteristics of study subjects are shown in **Table 1**. Echocardiographic examinations revealed that LVIDd and LVIDs sizes were wider before the transcatheter closure, and began to decrease gradually after one month and three months of closure. The NT-proBNP levels were higher before transcatheter closure than at one month and three months after transcatheter closure. Median NT-proBNP levels and left ventricular diameters before closure, and 1 month and 3 months after closure are shown in **Figure 1**. Median NT-proBNP levels were significantly decreased 3 months after closure. Also, subjects had significant weight gain at 3 months after closure, with a mean value of body weight was 22.75 (SD 11.23) kg. Distributions of NT-proBNP levels, LVIDd, and LVIDs are listed in **Table 2**.

The median NT-proBNP levels in PDA patients was higher than in VSD patients at all time points (Table 2) [PDA: 214 (range 30-2805) pg/mL before closure, 73.37 (range 15-323) pg/mL 1 month after, and 50.5 (range 5-369) pg/mL 3 months after PDA closure; VSD: 88.83 (range 12-226) pg/mL before closure, 54.5 (range 23-162) pg/mL 1 month after, and 28 (range 6-74) pg/mL 3 months after VSD closure].

Table 1. Characteristics of the study population

Characteristics	N=34
Mean age (SD), months	94.52 (55.9)
Sex, n (%)	
Male	19 (55.9)
Female	15 (44.1)
Congenital heart disease, n (%)	
PDA	24 (70.6)
VSD	10 (29.4)
Mean defect size (SD), mm	4.6 (1.4)
Mean body weight (SD), kg	
Before closure	20.07 (10.84)
1 month after closure	20.83 (10.73)
3 months after closure	31.86 (43.89)

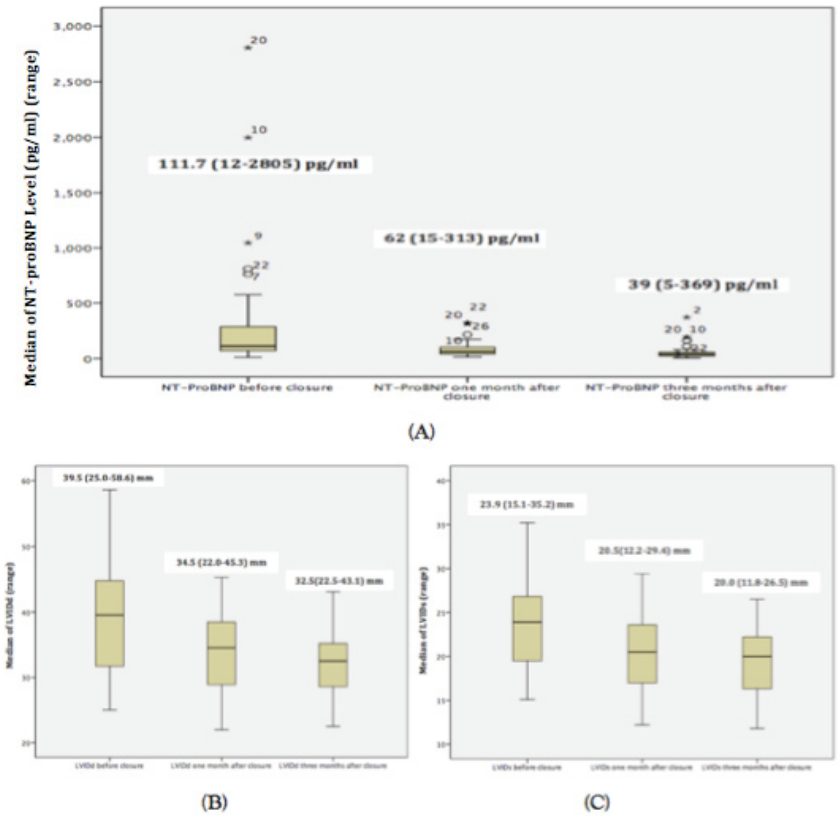

Figure 1. Distribution of NT-proBNP levels (A), diastolic (B) and systolic (C) end of left ventricle diameters

The median diastolic and systolic left ventricle diameters in PDA and VSD subjects before closure was wider than at 1 month and 3 months after closure that statistically significant, as shown in **Figure 2**. The median LVIDd and LVIDs based on type of congenital heart disease are shown in **Table 2**. In PDA subjects, median LVIDd values were 41.1 (range 25.0-58.6) mm before closure, 32.6 (22.0-45.3) mm 1 month after, and 30.9 (range 22.5-43.1) mm 3 months after closure, while median LVIDs values were 24.4 (range 15.1-35.2) mm before closure, 20.9 (range 12.2-29.4) mm 1 month after, and 19.5 (range 11.8-26.5) mm 3 months after closure. In the VSD subjects, median LVIDd values were 36.7 (range 30.8-45.6) mm before closure, 35.6 (range 28.9-45.0) mm 1 month after, and 33.7 (range 26.5-37.2) mm 3 months after closure, while median LVIDs values were 22.8 (range 18.4-27.5) mm before closure, 20.5 (range 15.3-23.6) mm 1 month after, and 20.0 (range 15.4-24.5) mm 3 months after closure.

Spearman's test revealed a moderate positive correlation between NT-proBNP levels and LVIDd (r=0.432; P=0.011), but no correlation with LVIDs at one month after closure (r=0.287; P=0.100). In addition, there were positive moderate correlations between changes of NT-proBNP levels and LVIDd (r=0.459; P=0.006) and LVIDs at three months after closure (r=0.563; P=0.001). Scatterplot charts of the correlations between NTproBNP levels and left ventricle diameters after one and three months of closure are shown in **Figure 3** and **Figure 4**.

Discussion

The PDA and VSD are two types of congenital heart disease that cause stretching of the left atrial and left ventricular heart muscle due to excessive volume load. The current choice of management for PDA and VSD, besides a surgical procedure, is transcatheter closure. The success rate of transcatheter closure is quite high, and it has become a feasible, effective, and safe procedure with good long-term outcomes based on previous studies.[14-23]

Atrial and ventricular hypertrophy are risk factors for all cardiovascular complications. Echocardiography is the gold standard examination to diagnose left ventricular hypertrophy, but it is impractical because it requires trained experts. This study was done to assess the use of NT-proBNP level to detect left ventricular hypertrophy. The NT-proBNP is a cardiac hormone produced by the ventricle, in response to volume load or pressure load. This hormone is a sensitive and specific indicator of ventricular function.[24,25] In our study, natriuretic peptide level was significantly higher before closure than after closure. At one month after closure, NT-proBNP level had declined and further decreased at three months after closure, due to decreased volume load after closure. High levels of NT-proBNP are reflective of excess left ventricular volume in asymptomatic cases with normal left ventricular diameter. The median left ventricular diameter during diastole and systole was wider before than after closure. The left ventricle diameter began

Table 2. Median NT-proBNP levels and median left ventricle systolic and diastolic diameters in PDA and VSD

Congenital heart disease	Median NT-proBNP level and left ventricle diameters (range)			P value
	Before closure	1 month after closure	3 month after closure	
PDA				
NT-proBNP, pg/mL	214 (30-2805)	73.37 (15-323)*	50.5 (5-369)**	0.000
LVIDd, mm	41.1(25.0-58.6)	32.6 (22.0-45.3)*	30.9(22.5-43.1)**	0.000
LVIDs, mm	24.4(15.1-35.2)	20.9 (12.2-29.4)*	19.5(11.8-26.5)**	0.000
VSD				
NT-proBNP, pg/mL	88.83(12-226)	54.5 (23-162)*	28 (6-74)**	0.000
LVIDd, mm	36.7(30.8-45.6)	35.6 (28.9-45.0)*	33.7(26.5-37.2)**	0.000
LVIDs, mm	22.8(18.4-27.5)	20.5 (15.3-23.6)*	20.0(15.4-24.5)**	0.000

Wilcoxon signed rank test
*NT-proBNP level significantly decreased one month after closure compared to before closure (P<0.05)
**NT-proBNP level significantly decreased three months after closure compared to before closure (P<0.05)

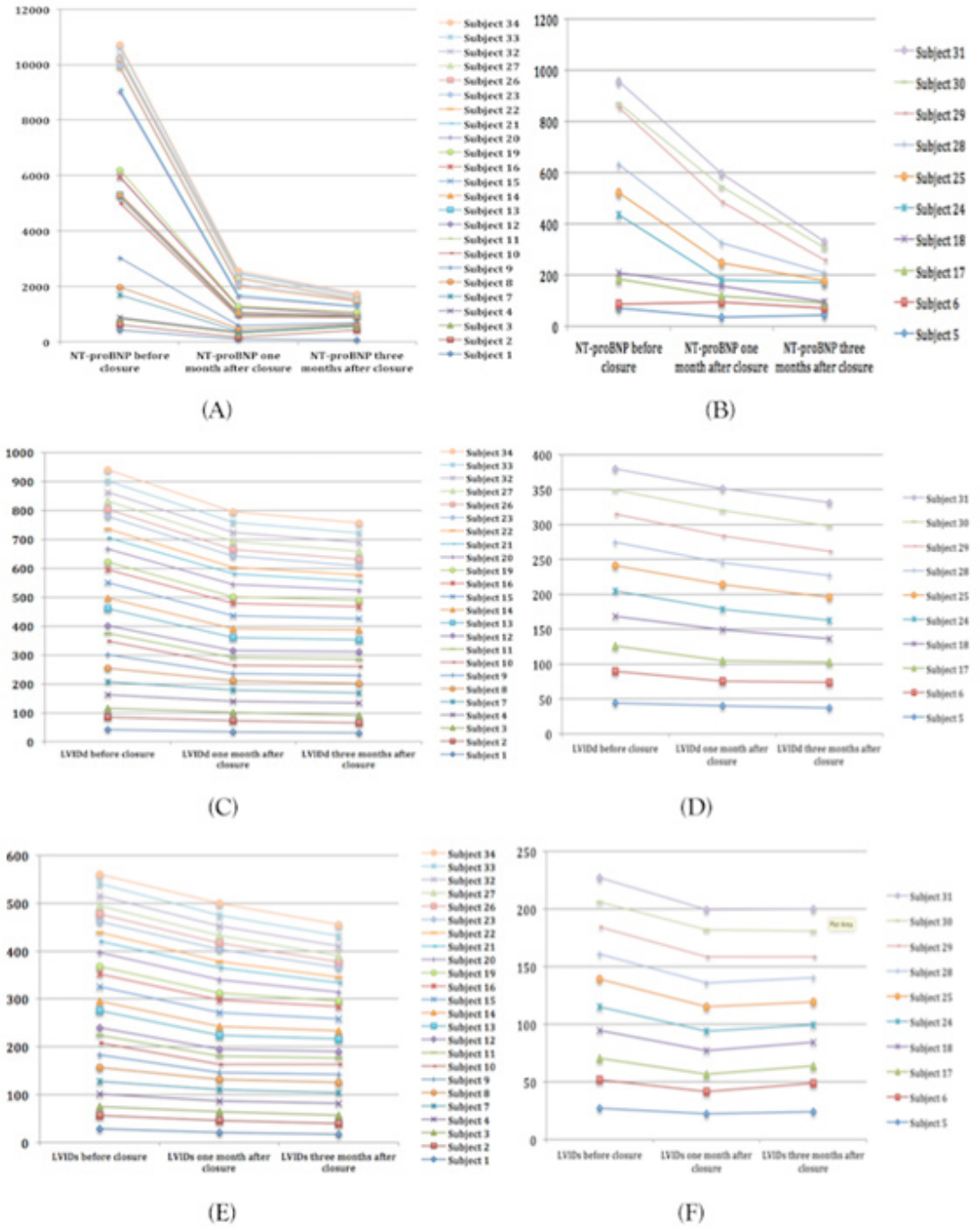

Figure 2. Distribution of NT-proBNP level in PDA subjects (A) and VSD subjects (B). Distribution of diastolic diameter in PDA subjects (C) and VSD subjects (D). Distribution of systolic diameter in PDA subjects (E) and VSD subjects (F).

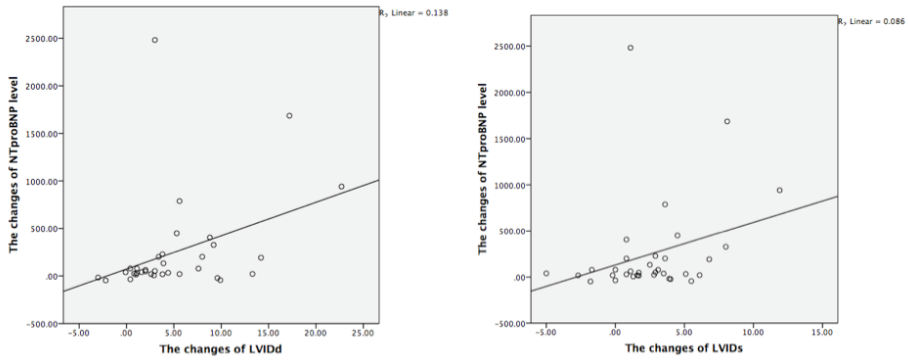

Figure 3. The correlation between NT-proBNP level and LVIDd (A) and LVID s (B) before and 1 month after closure

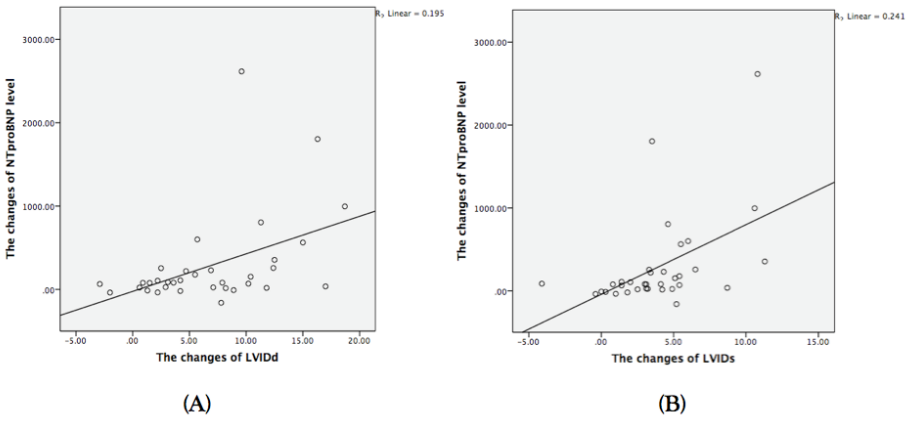

Figure 4. The correlation between NT-proBNP level and LVIDd (A) and LVIDs (B), before and 3 months after closure

to decrease gradually at one month and continued to decrease at three months after closure.

Increasing NT-proBNP levels reflect the stretching of the heart muscle, which tends to be higher in PDA subjects than in VSD subjects. Increased stretching in PDA patients may be the results of a different pathophysiological mechanism from that of VSD. Left ventricular hypertrophy in PDA and VSD occurs due to excessive volume load. The volume load occurs in the diastolic and systolic phases in PDA, but only in the systolic phase in VSD. This study was conducted to assess for possible correlations of NT-proBNP levels and left ventricular diameter before, one month after, and three months after closure. One month after closure, there was a moderate, positive correlation between NT-proBNP levels and diastolic left ventricular diameter, but no correlation with systolic left ventricular diameter. At three months after closure, a positive moderate correlation was observed between NT-proBNP levels and systolic and diastolic left ventricular diameters. Based on this study, three months after closure is a good time to assess NT-proBNP levels. This biomarker can used to determine the success of transcatheter closure. The results of our study were similar to those done by Elsharawy *et al.* and Eerola *et al.*, but we put more emphasis on the correlation between NT-proBNP and left ventricular diameter after closure. Elsharawy et al. noted a positive correlation between NT-proBNP levels and LVEDD, LVESD, and defect size.[10] Eerola *et al.* examined NT-proBNP levels with left ventricular dimensions at 1 day and 6 months

after transcatheter closure compared to a control group in PDA patients. They found an increase of NT-proBNP levels on the first day following the transcatheter closure, and decreased median diastolic volume and NT-proBNP levels after 6 months of transcermination.[11] Eerola *et al.* performed another study to investigate the association between peptide hormone levels and echocardiographic results, at 6 months and 12 months observation in ASD and coarctation of the aorta patients.[26] They reported decreased left ventricle diameters at six months after closure by about 20%, but in our study, left ventricular diameters decreased about 25% at three months after closure.

A limitation of this study was the small sample size, leading to difficulty in comparing between PDA and VSD. Also, our subjects were too heterogeneous with a wide age range as we did not exclude adolescent pubertal children. In addition, the monitoring period in this study was short, and should be continued to 12 months in order to assess for more significant reductions.

In conclusion, NT-proBNP level and left ventricular diameter are significantly correlated 3 months after closure. Decreased NT-proBNP levels are reflective of successful transcatheter closure.

Conflict of Interest

None declared.

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Original Article

Risk factors for relapse in pediatric nephrotic syndrome

Husein Albar, Fadel Bilondatu, Dasril Daud

Abstract

Background Nephrotic syndrome (NS) is the most common kidney disease in children and is characterized by edema, massive proteinuria, hypoalbuminemia, and hyperlipidemia. High relapse rate remains a major problem in the management of this syndrome.

Objective To identify risk factors for relapse in pediatric nephrotic syndrome.

Methods This study was carried out in the Wahidin Sudirohusodo Teaching Hospital in Makassar, South Sulawesi, Indonesia, from January to August 2017 using complete medical records of children diagnosed with NS. Subjects were divided into 2 groups: 1) relapsed NS or 2) non-relapsed NS. The following potential risk factors for relapse were analyzed using Chi-square test: age, sex, nutritional status, hypertension, serum creatinine level, and infection at the time of established diagnosis of NS.

Results A total of 142 children with NS who fulfilled the inclusion criteria aged 1.4 to 17.5 years were included in the study. Subjects were mostly boys (66.2%), with a male: female ratio of 1.95:1. The relapsed NS group had 80 cases (56.3%) and the non-relapsed NS group had 62 cases (43.7%). Statistical analysis revealed that nutritional status was a significant risk factor for relapse in pediatric nephrotic syndrome ($P<0.05$).

Conclusion Nutritional status is an independent risk factor for relapse in pediatric nephrotic syndrome. **[Paediatr Indones. 2018;58:238-42; doi: http://dx.doi.org/10.14238/pi58.5.2018.238-41].**

Keywords: *nephrotic syndrome; children; relapse risk factors*

Nephrotic syndrome (NS) is characterized by massive proteinuria (>40 mg/m^2/h), heavy hypoalbuminemia (<2.5 g/dL), edema, and usually accompanied by hypercholesterolemia >200 mg/dL, based on the *International Study of Kidney Disease in Children* (ISKDC) criteria.[1] Nephrotic syndrome is the most common kidney disease of children generally occurring in school-aged children less than 14 years of age.[1] Reports from the USA and UK showed that NS affects 2-7/100,000 children per year, with a prevalence of 12-16/100,000 children; whereas a report from Indonesia showed that NS affects 6/100,000 children under 4 years of age per year. The ratio of boys to girls was reported to be 2:1.[2-4]

The majority of children (90%) with idiopathic NS (INS) usually have minimal change NS (MCNS) on histopathologic findings, and 95% or more respond well to therapy with steroids.[5] However, INS is a chronic kidney disease generally tending to

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relapse. Remission is defined as absent (-) or trace (±) proteinuria (or proteinuria < 4 mg/m^2/hour) for three consecutive days in a week. Relapse was defined as urine protein ≥ 2+ (or proteinuria >40 mg/m^2/hour) for three consecutive days in a week in patients previously in remission.[3]

The International Study of Kidney Disease in children (ISKDC) originally reported a previous relapse rate of 60%,[1] but a later report showed a relapse rate increase of up to 76-90%, with a frequent relapse rate up to 50%.[3] There are several risk factors for relapse based on previous studies including age, sex, nutritional status, hypertension, creatinine levels, and infection at the time of diagnosis of NS. Therefore, if such risk factors in children with NS could be identified on admission to the hospital, better strategies for the management of pediatric nephrotic syndrome could be implemented in the future. This study was conducted to determine which variables were risk factors for the occurrence of relapse in pediatric nephrotic syndrome in Indonesia.

Methods

This study was conducted in the Department of Child Health, Wahidin Sudirohusodo Teaching Hospital, Makassar, South Sulawesi, Indonesia, from January to August 2017. We studied 142 children with INS hospitalized during the study period.

All patients who fulfilled the ISKDC criteria for the diagnosis of NS including massive proteinuria (or proteinuria >40 mg/m^2/hour or 50mg/kg/day; urinary protein/creatinine ratio of >2.0; or dipstick ≥ 2+), hypoalbuminemia (serum albumin < 2.5 g/dL), edema, and hypercholesterolemia (serum cholesterol >200 mg/dL) were further analyzed. Relapse was defined as urine protein ≥ 2+ (or proteinuria >40 mg/m^2/hour) for 3 consecutive days in a week in patients who had been in remission previously during the first 6 months of steroid therapy. Non-relapse was defined as absent (-) or trace (±) proteinuria (or proteinuria < 4 mg/m^2/hour) for 3 consecutive days in a week within 6 months of steroid therapy.

Nutritional status is the state of nutrition which is defined based on body weight parameter toward the body height based on *CDC-NCHS 2000* standard for children aged > 5 years and based on WHO for children aged ≤5 years. Hypertension is defined as systolic and/or diastolic blood pressure was above 95 percentage based on age and gender, for 3 consecutive times. Normal creatinine was define as the level of creatinine of 0.3 -1.2 mg/dL and increased creatinine if the level of creatinine is > 1.2 mg/dL. Infection is a disease that the patients had on admission beside the relapsed NS diagnosed, which was written on medical record, including pneumonia, diare, dermatological infection, and urinary tract infection. Drop out are those who did not come to the next control.

The subjects were divided into two groups: group I was comprised of children with INS who had relapsed after receiving up to 6 months of steroid therapy, whereas group II was comprised of children with INS who had sustained a state of remission for at least the first 6 months after receiving steroid therapy.

Patients with systemic and chronic diseases, congenital nephrotic syndrome, steroid resistance, and incomplete medical records (demographic and laboratory data) were excluded from the study. The following variables were recorded for all subjects: age, sex, height, body weight, history of infection, blood pressure, and laboratory findings such as serum protein, serum albumin, serum cholesterol, serum creatinine, complete blood count, as well as urinalysis, at the time of the NS diagnosis.

Data were presented by using univariate analysis on a categorical scale expressed as frequency with a corresponding percentage and the differences between groups was compared by using Chi-square test (bivariate analysis), using *SPSS* software. Variables that yielded a P value of < 0.05 by bivariate analysis were considered to be significant and subsequently further analyzed by multivariate analysis. The study was approved by the Research Ethics Committee of Wahidin Sudirohusodo Teaching Hospital, Makassar Indonesia.

Results

A total of 142 children with INS were included in this study. The majority of patients (66.2%) were 5 years of age or more, ranging from 1.4 to 17.5 years, with a mean age of 8.5 years. There were 94 (66.2%) boys and

48 (33.8%) girls, with a male: female ratio of 1.95:1. Eighty (56.3%) subjects belonged to the relapsed group and 62 patients (43.7%) to non-relapsed group. We noted that 56.3% of cases had normal nutritional status, 73.2% had normal blood pressure, and 63.4% had no evidence of infection at the time of diagnosis. Laboratory results showed that the majority of patients (90.1%) had normal creatinine levels and half of them (50.7%) had hematuria. The characteristics of subjects are shown in **Table 1**.

The possible risk factors for relapse were compared between the two groups. Chi-square test revealed no statistically significant difference in sex or age between the two groups. Also, no statistically significant differences between the relapsed and non-relapsed groups were seen in hypertension, infection, serum creatinine level, or hematuria. However, a statistically significant difference in subjects' nutritional status was observed between the relapsed and non-relapsed groups (P=0.02). Significantly greater percentages of undernourished and poorly nourished patients were in the relapsed group compared to the non-relapsed group (**Table 2**).

Table 1. Characteristics of subjects

Characteristics	(N=142)
Sex	
Male: female ratio, n (%)	94: 48 (66.2 : 33.8)
Age at diagnosis, n (%)	
≥ 5 years	94 (66.2)
< 5 years	48 (33.8)
Nutritional status, n (%)	
Normal	80 (56.3)
Undernourished	52 (36.6)
Poorly nourished	10 (7.0)
Hypertension, n (%)	
Yes	38 (26.8)
No	104 (73.2)
Creatinine level, n (%)	
Normal	128 (90.1)
Increased	14 (9.9)
Hematuria, n (%)	
Yes	72 (50.7)
No	70 (49.3)
Infection at diagnosis, n (%)	
Yes	52 (36.6)
No	90 (63.4)
Diagnosis, n (%)	
Relapsed	80 (56.3)
Non-relapsed	62 (43.7)

Table 2. Comparison of possible risk factors for relapse between the relapsed and non-relapsed groups

Variables	Group		P value
	Relapse (n = 80)	Non-relapse (n = 62)	
Sex, n (%)			
Male	56	48	0.277
Female	24	24	
Age at diagnosis, n (%)			
≥ 5 years	57	46	0.697
< 5 yearS	23	16	
Nutritional status, n (%)			
Normal	37	43	0.023
Undernourished	36	16	
Poorly nourished	7	3	
Hypertension, n (%)			
Yes	22	16	0.821
No	58	46	
Creatinine level, n (%)			
Normal	73	55	0.615
Increased	7	7	
Hematuria, n (%)			
Yes	41	31	0.883
No	39	49	
Infection, n (%)			
Yes	31	21	0.549
No	31	41	

Discussion

The frequency of relapse in idiopathic NS was 56.3% in our study, with a boy to girl ratio of 1.95:1. This ratio was similar to that of Constantinescu et al. who reported 1.8:1.6 But our frequency of relapse was lower than that of Mishra et al.7 in India and Subandiyah6 in Indonesia, who reported 59.3% and 65.9%, respectively.

In our study, the age of patients at the time of diagnosis was classified into either the ≤ 5 years of age group or >5 years of age group. A bivariate analysis revealed no statistically significant difference between the relapsed and non-relapsed groups in age at the time of diagnosis (P<0.697), similar to a study by Ali et al. (P=0.708).9 While the mechanism of INS remains unclear, it was hypothesized that INS may be caused by impaired T-cell function and the presence of abnormal T-cell clones producing chemical mediators as circulating glomerulotoxic lymphokines. These mediators increase permeability of the glomerular basement membrane, resulting in proteinuria. Abnormal T-cells are suspected to be cloned in the thymus, which is most active in childhood.[10]

We noted that the only statistically significant difference between the relapsed and non-relapsed groups was for nutritional status (P=0.023), with a higher percentage of poorly nourished subjects experiencing relapse. In contrast, Noer et al. found no statistically significant difference according to nutritional status of their patients. We found no statistically significant differences between the relapsed and non-relapsed groups in serum creatinine or hematuria levels, with P=0.615 and P=0.883, respectively. However, Sarker et al. found low levels of protein and serum albumin to be risk factors for frequent relapse.[3]

The limitations of our study were due to: 1) the patient data being taken retrospectively from medical records, 2) the recorded data being only six months after diagnosis, and 3) the non-relapsed group not further monitored after six months of steroid therapy. However, the strength of our study was that we excluded from further analysis those with incomplete medical record data as well as those who dropped out.

In conclusion, nutritional status of patients at the time of diagnosis may be used as risk factor for relapse in pediatric nephrotic syndrome. Clinicians should provide nutritional therapy if the NS patient is poorly nourished and reevaluate at least six months after steroid therapy. We suggest that further studies be performed without a six-month time limit to further assess other possible risk factors for relapse in pediatric nephrotic syndrome.

Conflict of Interest

None declared.

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Original Article

Low peripheral oxygen saturation as a risk factor for brain abscess in children with cyanotic CHD

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Abstract

Background Brain abscess is a severe infection of brain parenchyma, which occurs in 25-46% of cases of uncorrected cyanotic (CHD). Low arterial oxygen saturation is the main risk factor for brain abscess in children with cyanotic CHD, however, the arterial oxygen saturation test is invasive and not routinely done in our setting.

Objective To evaluate low peripheral oxygen saturation as a risk factor for brain abscess in children with cyanotic CHD.

Methods We conducted a matched, case-control study at Sardjito Hospital, Yogyakarta, for children aged less than 18 years with cyanotic CHD, from 2010-2016. Case subjects were children with brain abscess complications. The control group had only cyanotic CHD, and were matched for age and sex to the case group. During hospitalization due to the brain abscess complication in the case group, data regarding peripheral oxygen saturation, polycythemia, pneumonia, sepsis, dental caries and restricted pulmonary blood flow were collected and compared between both groups.

Results During the study period, 18 children with cyanotic CHD had brain abscesses. This group was compared to the control group of 36 children. Bivariate analysis revealed that the lowest level of peripheral oxygen saturation (OR 0.92; 95%CI 0.85 to 0.98; P=0.02) and dental caries (OR 3.3; 95%CI 1.01 to 11.18; P=0.04) were significant risk factors for brain abscess. However, in the multivariate analysis, the only statistically significant risk factor associated with brain abscess was the lowest level of peripheral oxygen saturation (OR 0.92; 95%CI 0.86 to 0.99; P=0.04).

Conclusion Low peripheral oxygen saturation is a significant risk factor for brain abscess development in children with cyanotic CHD. A decrease of 1% peripheral oxygen saturation may increase the risk of brain abscess by 8%. **[Paediatr Indones. 2018;58:252-6; doi: http://dx.doi.org/10.14238/pi58.5.2018.252-6]**.

Keywords: *brain abscess; cyanotic heart disease; peripheral oxygen saturationrisk factors*

Brain abscess is a severe infection of brain tissue that can be caused by bacteria, fungi, protozoa, and parasites.[1-3] The mortality rate caused by brain abscess is about 10%.[1]

Cyanotic congenital heart disease (CHD) with right-to-left shunt is one of the most frequent predisposing factors to brain abscess in children.[1,4] Of children with cyanotic CHD, 5-18% develop brain abscess complications.[1] Past studies from 1974 at Boston Children's Hospital and 1992 at the Neurological Institute and Heart Institute of Japan showed that the level of arterial oxygen saturation (SaO_2) in the abscess group was significantly lower than in the control group, in children with cyanotic CHD.[5,6]

Indonesia has an estimated 50,000 babies born with CHD every year. Like other developing countries, most patients with CHD seek medical treatment at later ages to have corrective cardiac surgery, which increases the risk of brain abscess complication. The oxygen saturation (SaO_2) level can be detected by

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arterial blood gas analysis, however, this examination is not done routinely in our setting, because it is invasive and expensive. Oxygen saturation can also be estimated by peripheral oxygen saturation level (SpO_2), as detected by pulse oximetry. The SpO_2 level considered to be a risk factor for brain abscess is still unclear. Hence, we aimed to evaluate low peripheral oxygen saturation as a risk factor for brain abscess in children with cyanotic CHD.

Methods

A matched, retrospective, case-control study was conducted in Dr Sardjito Hospital, Yogyakarta, from 2010 until 2016. Data were collected from medical records. Inclusion criteria for the case group were age between 1 month-18 years with diagnoses of brain abscess and cyanotic CHD, while the control group had only cyanotic CHD patients. The control group was matched for age and sex with the case group, at a ratio of 2:1. The exclusion criteria were incomplete medical records or cases of cyanotic CHD who had undergone corrective heart surgery.

Nutritional status was determined with WHO growth chart (normal: weight for age and weight for height between 2SD and -2SD, undernourished: weight for age or weight for height < -2SD, severe malnutrition: weight for age or weight for height < -3SD, and stunted: height for age <-2SD). Developmental status was determined using Denver II developmental screening test and was assesed in 4 major milestones aspect of which were, personal social, fine motor, language and gross motor aspect (normal: no developmental delay, delayed: delay in no more than 1 aspect, global developmental delay: delay in 2 or more aspect); and immunization status was classified as completed or not based on *National Immunization Programme* of Indonesia based on the age of subject.[8]

The SpO_2 levels recorded were the highest and lowest during hospitalization for every subject. The mean was calculated based on adding the highest and lowest SpO_2 values and dividing by two.

Data were collected and analyzed with *STATA 12.0*. Bivariate analysis using paired sample T-test or Wilcoxon signed rank test for numerical data, and P values <0.05 were considered to be statistically significant. Bivariate odds ratio (OR) for numerical data (SpO_2) was obtained by logistic regression with only SpO_2 as the predictor variable. Multivariate analysis using conditional logistic regression was done for the significant variables from the bivariate analysis. Results are reported as OR with 95% confidence interval (CI).

The study was approved by the Medical and Health Research Ethics Committee (MHREC), Universitas Gadjah Mada Medical School/Dr. Sardjito General Hospital, Yogyakarta.

Results

A total of 18 cases of brain abscess in children with cyanotic CHD were found in the 7-year study period. This case group was compared to the control group who had been matched for sex and age, at a 2:1 ratio (36 controls). The baseline characteristics of subjects are described in **Table 1**. Overall, the subjects' mean age was 7 years, and most subjects were male (83.3%). Stunting was frequently found in the case group (66.6%) and in the control group (50%).

The outcomes of the case group are described in **Table 2**. Abscess drainage was done only in 5 cases (27.7%). The abscess fluid culture results in subjects with abscess drainage were *Pasteurella pneumotropica*, *Eschericia coli*, and *Staphylococcus warneri*, while in 2 cases there were no growth. Antibiotics were given based on sensitivity and resistance results.

Bivariate analysis revealed that the mean lowest SpO_2 in the case group was significantly lower than that of the control group (P=0.02) (**Table 3**).

Bivariate and multivariate analyses were done to identify the confounding factors possibly contributing to the brain abscess incidence. Sepsis, pneumonia, and restricted pulmonary blood flow (PBF) could not be further analyzed because these variables each had a total of subjects below 5 in the case or control group.

Bivariate analyses revealed that lowest SpO_2 level during hospitalization was inversely associated with the occurrence of brain abscess (OR 0.93; 95%CI 0.86 to 0.99; P=0.023). Another significant variable for risk of brain abscess was dental caries (OR 3.3; 95%CI 1.01 to 11.18; P=0.04). Polycythemia had no significant correlation to brain abscess (OR 1.18;

Table 1. Baseline characteristics of subjects

Characteristics	With brain abscess (n=18)	Without brain abscess (n=36)
Mean age (SD), years	7.8 (4.4)	7.8 (4.4)
Sex		
Male, n (%)	15	30
Nutritional status, n		
Normal	6	14
Undernourished	6	15
Malnutrition	6	7
Stunted	12	18
Developmental status, n		
Normal	12	30
Delayed	3	2
GDD	3	4
Immunization status, n		
Complete based on NIP	11	35
Incomplete	7	1
Mean age at diagnosis of cyanotic CHD (SD), months	14.8 (22.4)	28.5 (41.5)
Type of cyanotic CHD, n		
TF	9	19
TGA	3	7
DORV	4	8
Pulmonal atresia	2	2
History of catheterization, n	6	27
History of spells, n	6	18
Heart failure, n	7	8

SD= standard deviation, GDD= global development delay, NIP=*National Immunization Programme,* TF=Tetralogy of Fallot, TGA=transposition of great arteries, DORV=double outlet of right ventricle

Table 2. Outcomes of cyanotic CHD subjects with brain abscess

Outcomes	With brain abscess (n=18)
Main symptoms, n	
Fever	14
Seizure	8
Vomiting	8
Headache	10
Neurological deficits	3
Decreased consciousness	1
Mean length of stay (SD), days	47.6 (24)
Brain abscess characteristics	
Location, n*	
Cerebellum	1
Thalamus	1
Brainstem	1
Fronto-parietal lobe	3
Parieto-occipital lobe	2
Parieto-temporal lobe	4
Occipital lobe	1
Parietal lobe	6
Number of abscesses, n	
Single	7
Multiple	11
Other main findings in head CT-scan, n	
Brain edema	8
Ventriculomegaly	8
Intracranial bleeding	4
Midline shift	4
Management, n	
Adequate antibiotics	7
Inadequate antibiotics	6
Abscess drainage	5
Outcomes, n	
Died	3
Lived with neurological sequelae	8
Lived without neurological sequelae	7
Neurological sequelae, n	
Seizure	1
Paresis	6
Seizure and paresis	1

*one subject had abcesses in both the parieto-occipital lobe and thalamus, hence counted as 2 subjects

Table 3. Peripheral oxygen saturation level as a risk factor of brain abscess in patient with cyanotic CHD

Risk factors	With brain abscess (n=18)	Without brain abscess (n=36)	P value *
Median of SpO_2 (IQ range)	73.5 (66-76)	75.5 (72-81)	0.27
Median highest SpO_2 (IQ range)	81.5 (78-87)	83.5 (78-88)	0.98
Mean lowest SpO_2 (SD)	63.0 (10.1)	70.3(7.6)	0.02

SpO_2=peripheral oxygen saturation, SD=standard deviation, IQ=interquartile, *=paired sample T-test/Wilcoxon signed rank

Table 4. Risk factor of brain abscess in children with cyanotic CHD

Risk factors	With brain abscess (n=18)	Without brain abscess (n=36)	Bivariate analysis		Multivariate analysis	
			OR (95%CI)	P value	aOR (95%CI)	P value
Mean lowest SpO2 level (SD)	63.0 (10.1)	70.3 (7.6)	0.92 (0.85 to 0.98)	0.02	0.92 (0.86 to 0.99)	0.04
Polycythemia, n	6	13	1.18 (0.37 to 3.75)	0.77	-	-
Sepsis, n	8	1	-	-	-	-
Pneumonia, n	2	0	-	-	-	-
Dental caries, n	10	9	3.3 (1.01 to 11.1)	0.04	2.7 (0.75 to 10.21)	0.12
Restricted PBF, n	16	36	-	-	-	-

SpO_2=peripheral oxygen saturation, SD=standard deviation, OR=odds ratio, aOR=adjusted odds ratio, CI=confidence interval, PBF=pulmonary blood flow

95%CI 0.37 to 3.75; P=0.77). Multivariate analysis on the significant variables in the bivariate analysis revealed that the only significant risk factor for brain abscess was the lowest SpO_2 level (OR 0.92; 95%CI 0.86 to 0.99; P=0.04) (**Table 4**).

Discussion

In a 7-year period, there were 18 subjects with brain abscess and cyanotic congenital heart disease. Subjects' mean age was 7 years and the youngest subject was 2 years of age. These data support the previous evidence which concluded that the brain abscess formation rarely happen on infants (<1 year old).[7] Bacteremia episodes in infants are extremely rare. Also, den-tation has been theorized as the most frequent source of intermittent bacteremia, so younger children with incomplete tooth growth may be less prone.[8]

The outcomes of our serial 18 brain abcess cases were as follows: 3/18 subjects died, 8/18 subjects lived with neurological sequelae and 7/18 subjects lived without neurological deficits. Similarly, a previous study in 2006 reported that 16% of subjects died, 53% lived with neurological sequelae, and 30% lived without neurological sequelae.[1]

Our study demonstrated that the main risk factor for brain abscess in children with cyanotic congenital heart disease was the low level of peripheral oxygen saturation recorded. Peripheral oxygen saturation measurement is non-invasive, and therefore, frequently used as an estimate of arterial oxygen saturation. Previous study concluded that the main risk factor was the low level of arterial oxygen saturation, however, there has been limited study on peripheral oxygen saturation as a risk factor of brain abscess. Fischbein *et al.* reported that the level of arterial oxygen saturation was a significant risk factor (P<0.01).[5] In addition, Takeshita et al. showed that the mean level of SaO_2 in 21 subjects with cyanotic congenital heart disease and brain abscess [67.2 (SD 12.5)%] was significantly lower than the control group [79.7 (SD 10.3)%], with P<0.01.[6] We also found that the lowest mean SpO_2 level in the abscess group [63.05 (SD 10.1) %] was significantly lower than in the group without brain abscess [70.38 (SD 7.6) %], with P=0.02.

We found no significant difference in SpO_2 for mean and median highest level between groups. This inconsistency might have been caused by the high variation in SpO_2 values recorded by pulse oximetry,

caused by certain conditions[9,10] that could not be controlled in this study, such as light (bright light directly to probe may affect the reading), shivering (movement may cause difficulty for the probe to pick up signal), pulse volume (low pulsatile in shock condition and arrhtythmia may also affect the reading), vasoconstriction, and also oxygen supplementation given to the subjects. The weakness of this study was that the data used were based on medical records, therefore, we do not know if such conditions occurred during the SpO_2 recording.

Other possible risk factors for brain abscess incidence were polycythemia, sepsis, pneumonia, dental caries, and restricted pulmonary blood flow. However, polycythemia was not a significant risk factor of brain abscess in this study (P=0.77), a finding similar to a previous study in which no hemoglobin or hematocrit level differences were noted between groups.[5,6]

There were 10/18 (55.5%) subjects with dental caries in the case group, but only 9 (25%) subjects with dental caries in the control group. Dental caries incidence was significantly different between groups, on bivariate analysis (P=0.04). High incidence of dental caries in patients with brain abscess suggests that dental infection might be a bacterial port d'entry to the blood circulation, leading to brain abscess formation.[11] However, this finding should be studied further by comparing the bacterial agents causing the brain abscess and the dental caries.

Multivariate analysis was also done for the significant variables from the bivariate analysis, revealing that only the lowest SpO_2 level consistently had a significant inverse association with the occurrence of brain abscess (P=0.04). The OR point estimate signifies that a 1% decrease of lowest SpO_2 level lowers the odds of developing a brain abscess by 0.93 (95%CI 0.86 to 0.99) times the patient's baseline odds.

A weakness of this study was the small sample size and retrospective design for which complete medical records are very important. The strength of this study was that it was the first of such studies in Yogyakarta, Indonesia. Our intital findings reported here provide basic data on evaluation of risk factors and outcomes of brain abscess in children with cyanotic congenital heart disease in Indonesia. In conclusion, low peripheral oxygen saturation is the main risk factor for brain abscess in children with cyanotic congenital heart disease.

Conflict of Interest

None declared.

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Original Article

Predictors for coronary artery dilatation in Kawasaki disease

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Abstract

Background Kawasaki disease (KD) is an acute, self-limited, febrile illness of unknown cause that predominantly affects children below 5 years of age. It has a high incidence of coronary complications such as aneurysms. The current treatment of choice is intravenous immunoglobulin, which is costly, with aspirin. Identifying the predictive factors for coronary artery dilatation or aneurysm is important in order to establish the indications for giving immunoglobulin, especially when resources are limited.

Objective To identify the predictors for the development of coronary artery dilatation in patients with Kawasaki disease

Methods This cross-sectional study was done between January 2003 and July 2013. Inclusion criteria were patients who fulfilled the *American Heart Association* criteria for acute Kawasaki disease, and had complete clinical, echocardiogram, and laboratory data [hemoglobin, leukocyte, platelet, albumin, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR)]. All of them received immunoglobulin and aspirin.

Results Of 667 KD patients, 275 met the inclusion criteria. There were 185 (67%) males. Subjects' ages varied between 1 to 157 months. The frequency of coronary artery dilatation at the acute phase was 33.3%. Multivariate analysis showed that >7-day duration of fever and hypoalbuminemia were significant predictive factors for coronary artery dilatation.

Conclusion Predictive factors for coronary artery dilatation are duration of fever over 7 days and hypoalbuminemia, while age, gender, hemoglobin level, leukocyte count, and platelet count are not. Frequency of coronary artery dilatation at the acute phase is 33.3%. **[Paediatr Indones. 2018;58:257-62; doi: http://dx.doi.org/10.14238/pi58.5.2018.257-62]**.

Keywords: *coronary dilatation; Kawasaki disease; predicting factor*

Kawasaki disease is an acute, self-limited, febrile illness of unknown cause that predominantly affects children below 5 years of age.[1] Coronary artery aneurysms occur in 15-25% of untreated cases, which may lead to myocardial infarction, sudden death, or ischemic heart disease.[2] The main current treatment to prevent coronary artery dilatation is high dose immunoglobulin and aspirin.

Considering the high cost of immunoglobulin, in cases with limited financial means and/or facilities, identifying risk factors for coronary artery dilatation may help physicians predict which patients are necessary to have immunoglobulin therapy. When the risk is high, then immediate treatment or referral would be mandatory.

Initial echocardiogram during the early days of the disease may not be useful to assess the need for immunoglobulin. Coronary artery dilatation is detectable from day 6 after onset, but commonly reaches its peak between 2-6 weeks from onset.[3-6] Furthermore,

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facilities and pediatric cardiologists are unavailable in most Indonesian hospitals. Therefore, it is of value to determine the predictors for coronary artery dilatation by other parameters that can been observed earlier.

Several scoring systems are available to assess the indications of giving immunoglobulin.[7-9] However, none are sensitive and specific enough to accurately detect the possibility of coronary aneurysm.[10] Thus, many experts in Japan who previously gave intravenous immunoglobulin only to the high risk patients, now give it to all Kawasaki patients in the acute phase.[11] Administering immunoglobulin to all KD patients in Indonesia would be financially prohibitive, and we do not know if the progression of the disease is the same in Indonesian patients.

It has been widely accepted that delayed administration of intravenous immunoglobulin is a major risk factor for coronary aneurysm.[1,11] Other risk factors are duration of fever,[7,12-15] age less than 1 year,[7-9,16,17] male sex,[9,17] and Asian/Hispanic race.[7,17] Laboratory examinations such as leukocytosis,[7-9] low albumin level,[9,16,18] high CRP level,[7-9] high ESR level,[7,13] anemia,[3,7] and thrombocytopenia[3,9] or thrombocytosis at acute phase[8] are also important risk factors.

Coronary artery dilatation was once determined using criteria from the Japanese Ministry of Health, but currently Z-score criteria is preferred.[1] The correlation between coronary artery dilatation and risk factors such as young age, male sex, leukocytosis, high neutrophil level, thrombocytopenia, high CRP level, high ESR level, low albumin, anemia, delayed treatment, and duration of fever using the Japanese Ministry of Health is inconsistent. In contrast, Z-score cutoff for coronary artery dilatation is relevant to young age, duration of onset to therapy, low IgM level, and low albumin level.[18] Until now, risk or predictive factors for coronary artery dilatation are diverse and sometimes contradict each other.

This study aimed to identify the predictors for the development of coronary artery dilatation in patients with Kawasaki disease.

Methods

This cross-sectional study using retrospective data was done between January 2003 and July 2013. Subjects were children with Kawasaki disease from 5 hospitals in the Jakarta area. Data of eligible subjects were taken from medical records, consisting of clinical presentation, therapy, laboratory studies (hemoglobin, leukocytes, platelets, albumin, CRP, and ESR) and echocardiography examination.

The minimum required sample size for studying coronary artery dilatation was estimated using an incidence of 20% and acceptable standard deviation of 5% ($N=Z\alpha 2PQ/d^2=1.962x0.2x0.8/0.052=246$). The minimum required sample size for risk factors of coronary dilatation was calculated based on the rule of thumb: >400 subjects (large), 200-400 subjects (intermediate), and <200 subjects (small).

Inclusion criteria were patients who fulfilled the *American Heart Association* diagnostic criteria for complete/incomplete KD in the acute phase,[1,10] were treated with immunoglobulin (2 g/kg BW) and oral aspirin (80-100 mg/kg), and had complete laboratory and echocardiogram data. Complete Kawasaki disease is diagnosed in the presence of fever for at least of 5 days with at least 4 of the 5 clinical findings: erythema of lips and tongue, bilateral conjunctivitis, rash, palmar/plantar erythema and cervical lymphadenopathy, while incomplete Kawasaki is diagnosed when fever is accompanied by less than 4 clinical findings in the presence of coronary artery dilatation or other laboratory criteria.[1] Subjects with congenital heart disease, history of other acquired heart disease, or incomplete/missing data were excluded. Independent variables were gender, age, duration of fever, hemoglobin, leukocytes, platelets, albumin, ESR, and CRP. The dependent variable was coronary artery dilatation. All clinical and echocardiographic examinations were done by the author (NA) before immunoglobulin and aspirin administration.

For assessment of coronary artery dilatation, we measured the lumen dimensions and used a z-score based on body surface area for the right, left main, and left anterior coronary arteries.[19] Coronary dilatation was defined as z-score ≥ 2.5 mm of any of the right, left main, or left anterior descending vessels. This study was approved by the Ethics and Research Committee of Universitas Indonesia Medical School.

Results

A total of 667 KD patients were found during the study period, 503 of whom were hospitalized during the acute phase. Coronary artery dilatation at this phase was seen in 168 patients (33.3%). Of the 503 subjects, 275 had complete medical records for risks factors (duration of fever, hemoglobin (Hb), leukocyte, platelets, ESR,CRP and serum albumin level). The majority of patients had complete Kawasaki disease (86.7%). There were 185 (67%) male and 90 (33%) female subjects. Subjects' ages ranged from 1 to 157 months. The majority of subjects were under 5 years (71%), with the highest incidence between 1 to 2 years.

Table 1 shows correlations between predictors and coronary artery dilatation. Bivariate analysis revealed that duration of fever, hemoglobin, platelet, ESR and serum albumin level had P values < 0.25. Subsequent multivariate analysis of these factors revealed that >7-day duration of fever and hypoalbuminemia were statistically significant as predictors for coronary artery dilatation (**Table 2**).

Discussion

Cardiovascular manifestations and complications are the major contributors to morbidity and mortality related to KD, with coronary aneurysms as the main complication. In our study, we use the term 'dilatation' rather than 'aneurysm,' as we measured at the acute stage, meaning the dilatation may be transient. Aneurysm is considered to be a sequela that persists beyond 30 days.[20] The long-term outcomes of these patients have been published elsewhere.[21]

The predictors for coronary artery dilatation in our study were duration of fever >7 days and hypoalbuminemia. We used the cut-off point of 7 days

Table 1. Correlations between predictors and coronary artery dilatation (bivariate analysis)

Dependent variables	Coronary dilatation (n=119)	No coronary dilatation (n=156)	OR (95%CI)	P value
Mean age (SD), months	39.2 (30.2)	37.5 (27.4)		0.62*
Gender, n			1.22 (0.73 to 2.04)	0.45**
Male	83	102		
Female	36	54		
Duration of fever, n			2.76 (1.66 to 4.58)	<0.001***
> 7 days	58	40		
≤ 7 days	61	116		
Mean Hb (SD), g/dL	10.63 (1.5)	10.86 (1.16)		0.15*
Mean leukocytes (SD), /µL	16,683 (7,653)	16,108 (5803)		0.48*
Mean platelets (SD), /µL	526,264 (207,582)	444,029 (161,868)		<0.001*
Mean ESR (SD), mm	76.4 (39.9)	69.5 (37.8)		0.15*
Mean CRP (SD), mg/L	92.9 (63.9)	93.5 (68.8)		0.94*
Mean albumin (SD), g/dL	3.25 (0.57)	3.47 (0.53)		0.001*

*unpaired T-test, ** Chi-square, Hb=hemoglobin

Table 2. Logistic regression test for coronary artery dilatation predictors (multivariate hypothesis test with cut-off P<0.25)

Predictor variables	Bivariate analysis		Multivariate analysis	
	OR (95%CI)	P value	OR (95%CI)	P value
Duration of fever > 7 days	2.76 (1.66 to 4.58)	<0.001	2.02 (1.15 to 3.53)	0.014
Hemoglobin level		0.151	1.06 (0.86 to 1.3)	0.610
Platelets count		<0.001	1 (1 to 1)	0.010
ESR		0.149	1 (0.99 to 1.01)	0.862
Serum albumin level		0.001	0.53 (0.32 to 0.87)	0.012

due to the majority of coronary artery dilatation onset occur in the late first week to early second week after disease onset. An epidemiological study by Belay *et al.* involving 3,115 subjects during a 10-year observation showed that predictors or risk factors for coronary dilatation were age < 1 year, age 9-17 years, male, as well as Asia-Pacific and Hispanic races.[17] However, laboratory parameters were not mentioned.

Many studies agreed that delayed immunoglobulin administration is a major contributing factor for coronary artery dilatation.[7,12-14] A study on 130 KD patients reported that risk factors for coronary artery dilatation were duration of fever >14 days or 10-13 days, plus 2 or more criteria such as age <1 year, Hb ≤10 gr/dL, leukocyte count ≥14,000/μL, and serum albumin ≤3.5 gr/dL.22 Hypoalbuminemia was a risk factor for coronary artery dilatation, similar to our findings. Other parameters such as hemoglobin level, ESR, and leukocyte counts were not associated with coronary artery dilatation, also similar to the results of our study. Nevertheless, duration of fever >14 days differed from our study.[22]

Harada *et al.* evaluated a scoring system for immunoglobulin administration, recommending it if 4 out of 7 criteria were fulfilled within 9 days of fever onset. Other risk factors for coronary dilatation were leukocyte count >12,000/μL, platelets count <350,000/μL, CRP >3+, hematocrit <35%, serum albumin <3.5 g/dL, age <12 months, and male sex.[9] However, the only similarity to our study was the hypoalbuminemia as a risk factor for coronary artery dilatation.

A previous study in 78 subjects with Kawasaki disease showed that CRP +6, age <1 year, and thrombocytosis were risk factors for coronary artery dilatation. Nakano recruited subjects on days 4 to 7 after fever onset, so fever itself was not stated to be a risk factor for coronary artery dilatation.[8] Another study used *z*-scores for measurements of the right coronary artery (RCA) and left anterior descending (LAD), without the left main coronary artery (LMCA), and concluded that risk factors for coronary dilatation were duration of fever, hypoalbuminemia, young age, and low immunoglobulin M level.[18] Fever and hypoalbuminemia were risk factors, similar to our findings.

In light of our findings, we suggest that every patient with fever > 7 days and hypoalbuminemia be referred to a medical center with echocardiography facilities and supported a bya pediatric cardiologist. If referral is not possible, it is advisable to immediately give immunoglobulin and aspirin to reduce the possibility of coronary artery dilatation. This recommendation can be applied generally for all Kawasaki patients regardless of their risk factor status.

Duration of fever before immunoglobulin administration is a risk factor for coronary artery dilatation due to progressing inflammation. The pathophysiology of hypoalbuminemia and coronary dilatation is unclear. During acute phase, vascular endothelial growth factor (VEGF) plays a role in increasing vascular permeability,[23] which leads to vascular leakage and results in hypoalbuminemia and edema.[24] Therefore, low serum albumin is a marker for high VEGF. Increased vascular permeability due to VEGF also causes inflammatory cells to enter the intima via coronary artery endothelium, resulting in intimal hyperplasia and proliferation of smooth muscle cells.[25]

In a developing country such as Indonesia, where immunoglobulin cost is high while purchasing power is low, careful selection of patients who receive intravenous immunoglobulin based on predictors of coronary artery dilatation would be quite prudent, especially for those with limited funding.

However, it is not easy to accurately determine risk of coronary artery involvement, although some laboratory markers may provide helpful information for parental counseling and clinical follow up. Future identification of novel biomarkers and host predispositions may improve our knowledge of coronary artery risk factors and help personalize therapy for Kawasaki disease.

In conclusion, the predictors for coronary artery dilatation are duration of fever over 7 days and hypoalbuminemia, while age, gender, hemoglobin level, platelets count, leukocytes count, ESR, and CRP are not. The frequency of coronary dilatation at the acute phase of KD is 33.3%. All patients should be treated with immunoglobulin before day 7 of onset and those with hypoalbuminemia should be treated immediately to prevent coronary complications. When resources are limited, those with hypoalbuminemia and fever over 7 days should be prioritized for immunoglobulin treatment.

Conflict of Interest

None declared.

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Original Article

Liver iron overload and hepatic function in children with thalassemia major

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Abstract

Background Routine blood transfusions and increased intestinal iron absorption lead to iron accumulation in various organs, especially the liver. To date, T2-star magnetic resonance imaging (T2*MRI) is a valuable tool to evaluate iron level in organs.

Objective To assess the degree of liver iron overload among children with thalassemia major (TM) and its possible correlations with hepatic function laboratory values.

Methods This cross-sectional study was conducted in Cipto Mangunkusumo Hospital. The degree of liver iron overload was evaluated by T2*MRI. Assessments of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, and bilirubin levels were done to evaluate liver function.

Results A total of 291 TM children were included in this study. The mean age of subjects was 12 years. Most of the subjects were diagnosed as β-thalassemia homozygote (54.6%) and β-thalassemia/HbE (41.2%). Deferiprone (DFP) was the most commonly used iron chelator. Less than 10% of the subjects had normal liver iron deposition. The AST and ALT values increased proportionally with the severity of liver iron overload, with significant, moderately negative correlation coefficients (r=-0.388 and -0.434, respectively). However, albumin level decreased proportionally with the severity of liver iron overload, with a significant, moderately positive correlation coefficient (r=0.323). Liver T2* MRI had no significant correlations with direct, indirect, and ratio of direct/total bilirubin levels.

Conclusion Most of the children with TM have mild to severe liver iron overload. Liver T2* MRI has significant, moderate correlations with AST, ALT, and albumin values. Bilirubin level has no correlation with T2* MRI. Our findings suggest that monitoring of AST, ALT, and albumin levels is important because they may reflect the severity of liver iron overload. However, they should not be used as the only predictors of iron overload. **[Paediatr Indones. 2018;58:233-7; doi: http://dx.doi.org/10.14238/pi58.5.2018.233-7].**

Keywords: *thalassemia; liver iron overload; MRI; hepatic function test*

Thalassemia is an inherited blood disorder characterized by decreased or absent globin chains. It is the most common single gene disorder worldwide and is mostly inherited in an autosomal recessive pattern. There are two main types of thalassemia, α- and β-thalassemia. The combination of thalassemia and hemoglobin variant has a high prevalence in the population.[1]

Thalassemia major (TM) is the most severe form of thalassemia. The two main treatments for TM are routine blood transfusions and iron chelation therapy. The iron from blood transfusions accumulates in organs. A state of chronic anemia causes increased iron absorption in the gastrointestinal system. Subsequently, these two conditions lead to iron overload in various organs, which may induce cell damage.[2]

Among various organs, the liver has the highest capacity to store excess iron in the body and is very prone to damage by iron toxicity.[3] Therefore,

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evaluation of iron deposition in the liver is crucial in TM patients. One of the best techniques to evaluate liver iron deposition is T2* MRI because it is non-invasive, reproducible, and accurate.[4] In this study, we aimed to evaluate the degree of liver iron overload in pediatric thalassemia major patients, as well as its correlation with laboratory values of hepatic function.

Methods

This cross-sectional study was done in Cipto Mangunkusumo Hospital. Iron deposition in the liver was assessed by T2* gradient echo (GRE) sequence MRI 1.5 Tesla (Siemens Avanto, Germany). Liver T2* value was acquired after scanning the center of the liver at 12 different echo times (1.3-23 ms) and analyzed using *CMRtoolsTM* software (Thalassemia-Tools, London, United Kingdom). The degree of liver iron overload was determined based on T2* MRI value: normal >6.3 ms, mild 2.7-6.3 ms, moderate 1.4-2.7 ms, and severe <1.4 ms.5 Blood specimens were collected to evaluate laboratory values of liver function: AST, ALT, albumin, direct bilirubin, and indirect bilirubin. The normal reference laboratory values for these assessments were as follows: AST <27 U/L; ALT <23 U/L; albumin 3.2-4.4 g/dL; direct bilirubin <0.3 g/dL; and indirect bilirubin 0.3-0.9 g/dL.

Results

A total of 291 TM children were included in this study. The mean age of subjects was 12 (SD 1.64) years. There was no significant difference between the number of male and female subjects. Most subjects were diagnosed with β-thalassemia (54.6%) and β-thalassemia/HbE (41.2%). Deferiprone (DFP) was the most common iron chelator used, followed by deferasirox (DFX), and a combination of DFP-DFX. None of the subjects used deferoxamine (DFO) as a single iron chelator. More than 90% of subjects had mild to severe liver iron overload (**Table 1**).

Table 2 shows the laboratory values of hepatic function in different stages of liver iron overload.

Table 1. Demographic characteristics of subjects

Variables	(N=291)
Mean age, years (SD)	12 (1.64)
Sex, n (%)	
Male	156 (53.6)
Female	135 (46.4)
Type of thalassemia, n (%)	
α-thalassemia	10 (3.4)
β-thalassemia	159 (54.6)
β-thalassemia/HbE	120 (41.2)
α-β-thalassemia/HbE	2 (0.8)
Iron chelator, n (%)	
Monotherapy	
DFO	0 (0)
DFP	191 (65.6)
DFX	40 (13.7)
Combination therapy	
DFO+DFP	17 (5.9)
DFO+DFX	5 (1.7)
DFP+DFX	34 (11.7)
No chelation	4 (1.4)
Degree of liver iron overload, n (%)	
Normal	27 (9.3)
Mild	182 (28.2)
Moderate	01 (34.7)
Severe	81 (27.8)

The AST tended to increase proportionally with the degree of liver iron deposition. The same result was observed for ALT values. However, mean albumin level in normal liver was the highest (4.66 g/dL), and decreased with increasing severity of iron overload. The mean direct and indirect bilirubin values were lower in mild and moderate liver iron overload, compared to normal liver. However, these mean values increased in the severe iron overload group. There was only a slight difference in direct bilirubin between the mild and moderate liver iron overload groups. The mean of direct/total bilirubin ratio was almost equal for all degrees of liver iron overload (0.29-0.33).

Table 3 and **Figure 1** show significant moderate correlations between liver T2* MRI and AST, ALT, and albumin ($P<0.05$ for all). The ALT value showed the strongest correlation ($r=-0.434$), followed by AST ($r=-0.388$), and albumin ($r=0.323$). However, the former two were negative and the third was a positive correlation. There was no significant correlation between liver T2* MRI and bilirubin level.

The scatterplots of the liver function test values are presented in **Figure 1**.

Table 2. Liver function indicators among different degrees of liver iron overload

Variables	Degree of liver iron overload			
	Normal (n=27)	Mild (n=82)	Moderate (n=101)	Severe (n=81)
Mean AST (SD), U/L	29.55 (11.44)	28.89 (18.04)	33.10 (16.30)	44.72 (22.35)
Mean ALT (SD), U/L	20.85 (17.74)	21.44 (22.04)	32.26 (23.60)	46.58 (37.93)
Mean albumin (SD), g/dL	4.66 (0.36)	4.55 (0.28)	4.46 (0.34)	4.32 (0.33)
Mean direct bilirubin (SD), mg/dL	0.53 (0.14)	0.42 (0.15)	0.43 (0.16)	0.59 (0.43)
Mean indirect bilirubin (SD), mg/dL	1.53 (1.03)	1.08 (0.55)	0.91 (0.39)	1.21 (0.52)
Mean direct/total bilirubin ratio (SD)	0.31 (0.08)	0.29 (0.06)	0.33 (0.05)	0.32 (0.08)

Table 3. Correlation coefficient between liver T2* MRI and liver function tests

Indicator	r	P value
AST	- 0.388	0.001
ALT	- 0.434	0.001
Albumin	0.323	0.002
Direct bilirubin	0.032	0.735
Indirect bilirubin	0.109	0.248
Direct/total bilirubin ratio	- 0.146	0.120

Discussion

The liver is the most important organ for iron metabolism in the body. It has three essential functions: 1) it is the primary site for production of iron-binding proteins, including transferrin, which is the major serum iron-binding protein that maintains systemic iron balance; 2) it is also the major storage site for iron excess, facilitated by liver ferritin that can store up to 4,500 atoms of iron; and 3) it controls the mobilization of iron from storage site to circulation for metabolism.[3]

Liver contains about 70% of total body iron. Therefore, liver iron concentration may reflect the total iron body contents and be useful for monitoring response to therapy. This fact gives rise to another consequence: the liver is the organ most damaged by iron, compared to other organs in the body.[6] Accumulated iron in cells leads to the production of reactive oxygen species (ROS), primarily by Fenton reaction. Ferrous iron catalyzes the decomposition of hydrogen peroxide, forming hydroxyl radicals, which are the most toxic ROS. Hydroxyl radicals target both carbohydrate, protein, and nucleic acids. Long term iron toxicity leads to cell death, fibrosis, and carcinogenesis.[7,8]

Several biochemical markers are widely known to reflect hepatic function: AST, ALT, albumin, and serum bilirubin. In our study, increased AST and ALT may reflect the severity of liver iron overload. The AST and ALT are cytoplasmic enzymes that catalyze the transaminase reaction in liver. Any kind of hepatocellular injury may disrupt cellular membrane permeability and cause leakage of transaminase enzymes into the extracellular compartment. Subsequently, elevated enzyme activity can be detected in the blood.[9,10]

Another interesting finding in this study was that mean AST values were observed to be within normal range in all iron overload groups, whereas mean ALT values were normal only in the normal and mild liver iron overload groups. This finding may have been due to ALT's greater specificity for liver than that of AST. The highest concentration of AST is found in heart, compared to other organs such as liver, skeletal muscle, and kidney. However, ALT is primarily found in liver, compared to other organs.[9,11]

Our study demonstrated that albumin synthesis decreased as the severity of liver iron overload increased. The coefficient correlation between liver T2* MRI value and albumin was positive, unlike the transaminases. During albumin metabolism, only small portions of synthesized albumin are stored in liver, while the majority is released into the bloodstream. Any hepatocyte injury may cause decreased production of albumin, which can be then detected in blood.[12,13]

We did not find any significant correlations between liver T2* value and direct, indirect, or direct/total bilirubin ratio. Almost all subjects in this study had abnormal increased total bilirubin value (>1.2 mg/dL), with direct/total bilirubin ratio of 0.3. This finding

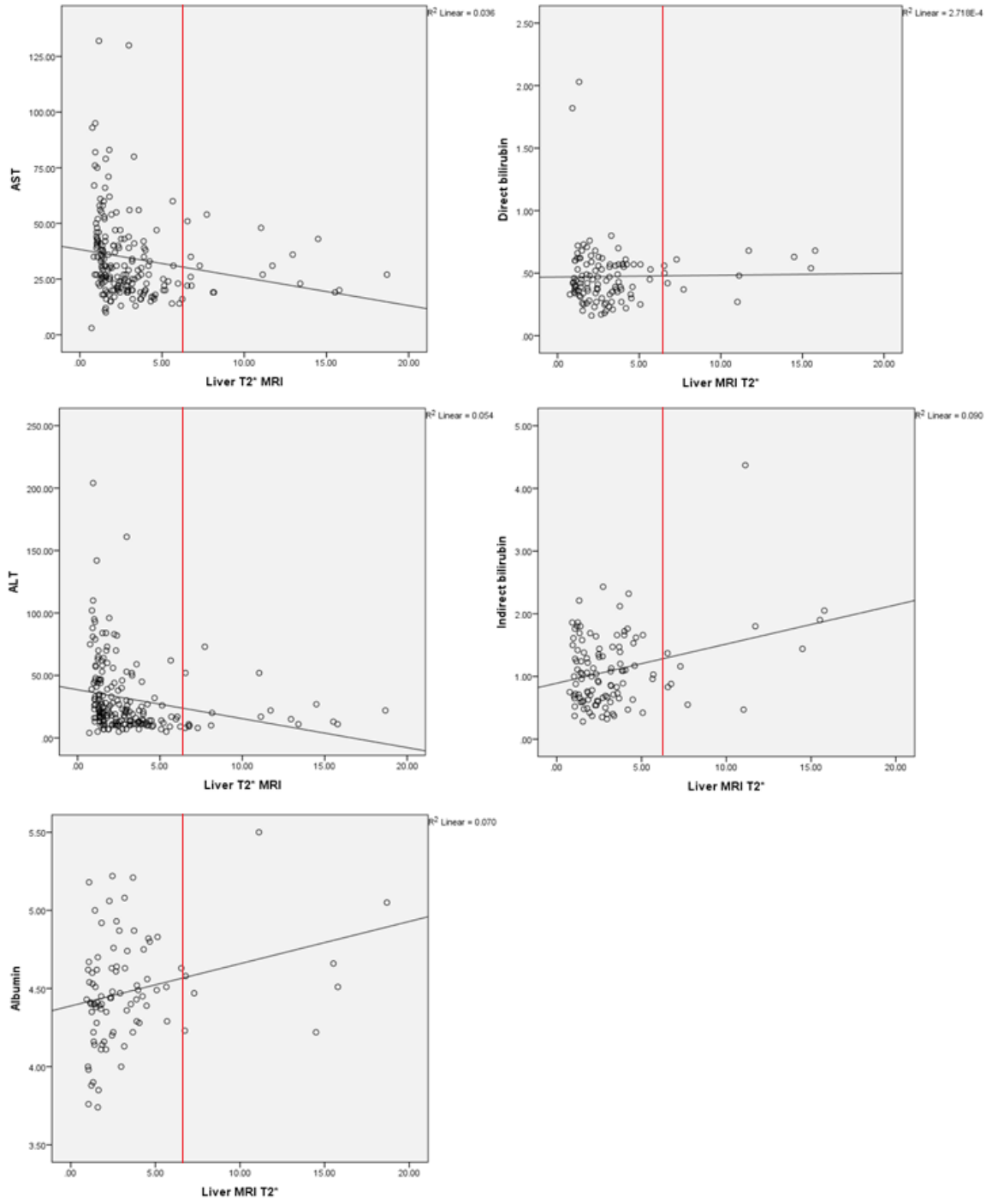

Figure 1. Scatterplots between liver MRI T2* value vs. hepatic function laboratory values (T2* value on the right side of red line is categorized as normal)

means that most of the subjects were in a conjugated hyperbilirubinemic state, regardless of the degree of iron overload. Conjugated hyperbilirubinemia in thalassemia subjects may be caused by hepatocellular damage due to iron toxicity.[9,14]

This study demonstrates that most children with TM have mild to severe liver iron overload. There are significant, negative moderate correlations between liver T2* values and AST and ALT, as well as a significant, positive moderate correlation with albumin. Bilirubin level has no correlation with T2* value. Our findings suggest that monitoring of AST, ALT, and albumin levels is important because they may reflect the severity of liver iron overload. However, they should not be used as the sole predictors of liver iron overload.

Conflict of interest

None declared.

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Original Article

Preterm infant physiological responses to music therapy: a systematic review

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Abstract

Background Prematurity is still the leading cause of mortality and morbidity in neonates. The premature change of the environment causes stress, which leads to hemodynamic instability. Music therapy may have a positive impact on hemodynamic parameters of preterm infants in the NICU.

Objective To evaluate preterm infants' physiological responses to music therapy in NICU setting.

Methods A systematic review was performed in 12 electronic databases from March 2000 - April 2018. Our review included all English language publications on parallel or crossover RCTs of music therapy versus standard care or placebo in preterm infants. The outcomes were physiological indicators [heart rate (HR), respiratory rate (RR), and oxygen saturation (SaO_2)]. Risk of bias was assessed using the revised Cochrane risk of bias tool for randomized trials (*RoB 2.0*).

Results The search yielded 20 articles on 1,148 preterm infants of gestational age 28 and 37 weeks, who received recorded music, recorded maternal/male voice or lullaby, or live music interventions in the NICU with intensity of 30-76 dB. Recorded music improved all outcomes in 6, 6, and 4 of 16 studies for HR, RR, and SaO_2, respectively. Seven studies used classical music as melodic elements. However, eight studies showed no significant results on all outcomes.

Conclusion Despite the finding that music interventions demonstrate promising results in some studies, the variation in quality of the studies, age groups, outcome measures, as well as type and timing of the interventions across the studies make it difficult to draw overall conclusions about the effects of music in preterm infants. **[Paediatr Indones. 2018;58:242-51; doi: http://dx.doi.org/10.14238/pi58.5.2018.242-51**].

Keywords: *music therapy; physiological response; preterm infants*

The World Health Organization (WHO) defines preterm birth as all births before 37 complete weeks of gestation or fewer than 259 days since the first day of a woman's last menstrual period. The annual national preterm birth rates from 1990 to 2010 for 65 countries in Europe, America, and Australasia showed increasing absolute numbers, suggesting an increasing burden of preterm birth.[1] In 2010, the estimated preterm birth rate was 11.1% of 135 million live births worldwide.[2] Furthermore, Indonesia is included among 10 countries with the highest numbers of estimated preterm birth and rates of 15% or more.[1]

Preterm birth is a major cause of death and long-term loss of human potential amongst survivors around the world. Additionally, preterm birth is also a direct cause of 35% of all neonatal deaths and the second most common cause of under-5 deaths, after pneumonia.[1] Preterm birth also increases the risk of dying due to other causes, especially neonatal infections. Thus, preterm birth is considered to be a risk factor for at least 50% of all neonatal deaths.[3]

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While preterm birth is well known for causing a high mortality rate, it is also associated with injury to many organ systems. Therefore, special care of preterm infants is crucial in the NICU. Nonetheless, the NICU itself is a stressful environment, as some infants must undergo painful events several times a day. Such exposure to a great number of stressors in the NICU has been associated with brain development issues in preterm infants.[4]

Variations in heart rate, blood pressure, oxygen saturation, and breathing patterns are the most frequently used physiological indicators of pain. Pain causes an increase in heart rate and blood pressure, a decrease in oxygen saturation, and more rapid, shallow, or irregular breathing.[5] In response to pain, ATP degradation increases due to energy expenditure through behavioral and physiological reactions to pain, such as crying, facial grimacing, flailing, and tachycardia. Moreover, oxidative stress also increases due to painful stimuli, resulting in additional ATP utilization. Consequently, these processes could lead to energy deficits.[6] One of the most important effects of energy deficit in preterm infants is low weight gain, which can affect brain development, and lead to poor growth and development.[7] Therefore, controlling pain-induced physiological responses is key to preventing poor development in preterm infants. Moreover, supportive therapy such as oxygen supplementation, fluid maintenance, and mechanical ventilation may have detrimental side effects when not used properly. Preterm infants can possibly suffer from ventilator-induced lung injury,[8] bronchopulmonary dysplasia,[9] retinopathy of prematurity,[10] and other catastrophic problems.

As an alternative solution, music therapy is often introduced to stabilize effective hemodynamic parameters in preterm infants, as it is easy to implement, non–invasive, relatively low-cost, and has no known side effects. While some studies showed promising results, others led to contradictory conclusions. Therefore, this systematic review was done with the aim of evaluating preterm infants' physiological response to music therapy.

Methods

This systematic review was conducted according to the *Preferred Reporting Items for Systematic reviews and Meta-Analysis* (PRISMA) guidelines. Relevant studies were identified in twelve online databases from March 2000 - April 2018. **Figure 1** shows the overview of the study identification process using a PRISMA flowchart. We searched the *Cochrane Database for Systematic and Complete Reviews* to identify systematic reviews and/or meta-analyses, followed by manual search of reference lists to identify relevant articles that were not identified by the search engines. The authors selected potential articles by screening titles and abstracts independently from each other. After accounting for duplication, we reviewed the titles and corresponding abstracts of all studies to identify articles that met the inclusion criteria. Thus, the full texts of all potentially relevant studies were reviewed to determine final study selection.

Inclusion criteria were all studies that investigated the effects of music therapy on physiological responses [heart rate (HR), respiratory rate (RR), or oxygen saturation (SaO_2)] in preterm infants (<37 weeks' gestation) that were published in English. Study designs were parallel or crossover randomized clinical trials of music therapy versus standard care, or placebo. Music therapy was given in the form of recorded music, recorded maternal/male voice or lullaby, or live music intervention in the NICU. Music was defined as intentional sound with pleasing harmonies, dynamics, rhythm, tempo, and volume. Instrumental music played by recorder or audio player in/outside the incubator was defined as recorded music; maternal voice or lullaby was described separately.[11-13] Live music was defined as singing lullabies and/or accompanied by instrument music, which was played live. Studies that identified the effects of music therapy on intrauterine life were excluded. Furthermore, conference proceedings, abstracts, review articles (systematic and narrative), case series or case studies, editorials, theses, dissertations, commentaries, and opinion-based papers were also excluded.

From each study, we extracted the study design, subjects' characteristics, physiological outcomes (HR, RR, SaO_2), and compared the results between the intervention and control groups. The results are presented as the characteristics of music intervention (**Table 1**). The results of music therapy are shown in **Table 2**. The quality of included studies was assessed by the *Grading of Recommendations Assessment, Development and Evaluation* (GRADE) tool (**Table 3**).

Risk of bias was assessed using the revised Cochrane risk of bias tool for randomized trials (*RoB 2.0*), as shown in **Table 4**. There were five bias domains: randomization process, deviations from intended interventions, missing outcome data, outcome measurement, and selection bias. Overall bias of each articles were classified as low, some concerns, and high risk.

Table 1. Characteristics of music intervention

Author (year)	Music selection	Type of output	Intensity (dB)	Duration of study, length, and frequency of intervention	Timing of intervention
Butt (2000)	Brahms' Lullaby and recorded acapella version	Speakers	76	NR, 10 minutes, once daily	After heel lance procedure
Calabro (2003)	Lullabies (Brahms and Sandman)	Speaker, located 15–20 cm from PI	60–70	4 days, 45 minutes, once daily	NR
Arnon (2006)	Live music and recorded music	Live music 1–2 m from bed. Recorded music: 2 speakers 1 m from bed	55–70	3 consecutive days, 30 minutes, once daily	1 hour after feeding
Lai (2006)	Lullabies (Western vocal, instrumental lullaby, aboriginal Taiwanese lullaby)	Speaker	NR	3 days, 60 minutes, once daily	1 hour after feeding
Johnston (2007)	Recorded mother's voice	Portable cassette tape player	60–70	2 days, 10 minutes, 3 times daily	After feedings
Whipple (2008)	Pacifier–activated lullaby (PAL)	PAL, 6 inches from infant's head, bilaterally	65	1 day, 10 minutes, only once	During heel stick procedure
Cassidy (2009)	Lullaby and classic music (Mozart)	Speaker (in incubator)	65–75	5 days, 40 minutes, once daily	NR
Farhat (2010)	Lullaby music (Iranian females vocalists)	Headphones	60–65	8 days, 20 minutes, once daily	½-1 hour after feeding and diaper change
Schlez (2011)	Live harp music therapy a blend of Eastern and Western melody	Distance 1–2 m	50–65	Alternating 3–5 days apart, 30 minutes, once daily	30 minutes after feeding, afternoon
Amini (2012)	Lullaby music (Iranian lullaby), classical music (Mozart sonata K448)	Two speakers in the corners of the incubator (30 cm from baby's ears)	45–50	6 days (2 days for each intervention and control), 20 minutes	1 hour after feeding
Alipour (2013)	Lullaby music	Headphones	50–60	20 minutes	30 minutes after the last feeding and nursing care
Auto (2013)	Recorded soft classical songs with low range, simple and direct rhythm (e.g., Mozart's)	NR	NR	Seven consecutive days of a week	Afternoon
Loewy (2013)	Live singing lullaby; live application of the Lullaby Ocean Disc; Gato Box (entrained live heartbeat sound)	Portholes of the incubators, isolettes or at bassinette side at the infants' midline	55–65	3 interventions and control per week within 2 week period	Either morning or afternoon
Arnon (2014)	Live maternal singing	Live intervention	60–70	2 days, 20 minutes, once daily	30 minutes after feeding
Aydin (2014)	Classical Turkish music	Two speakers were put in the direction of infant's toes within distance 30 cm	45 (max)	3 days per week, 30 minutes, once daily	Afternoon
Dearn (2014)	Brahms' lullaby	Microspeaker, 30 cm from infant's head	45–65	1 day, 12 minutes, only once	Morning and afternoon
Jabraelli (2016)	Brahm's lullaby and recorded mother's lullaby	NR	65–70	3 consecutive days, 15 minutes, once daily	Between 10 am –7 pm
Taheri (2016)	Recorded male lullaby	Headphones	50–60	3 days, 40 minutes, once daily	Same noon time
Wirth (2016)	Recorded lullabies and maternal voice group	Speaker, 20 cm from the infants' ears	55–65	14 days, 30 minutes, once daily	Between 8–9 pm
Caparros-Gonzales (2017)	Relaxing tune by Melomics computer system	Speaker, located 20 cm from infant's left ear	30–50	3 days, 20 minutes intervention, 3 times daily	In the morning (9-10 am), afternoon (2-3 pm), evening (9-10 pm)

PI: preterm infants; NR: not recorded

Results

The search strategy using MeSH terms (music, preterm infants, and physiologic responses) yielded 1,481 records. Further manuscript evaluation included screening for duplication, inclusion, and exclusion criteria (**Figure 1**).

Twenty articles were included, of which 12 were parallel and 8 were crossover randomized controlled trials. A total of 1,148 preterm infants between 28 and 37 weeks' gestational age admitted to the NICU either received recorded music, recorded maternal/male voice or lullaby, or live music interventions. All studies were published from 2000 to April 2018. Four studies investigated the effects of live music intervention, sixteen studies used recorded music, and three studies explored recorded maternal/male lullabies. Furthermore, three studies also compared two kinds of intervention groups (**Table 2**).

Four studies used live music intervention consisting of one live harp melody, one live wordless lullaby, one live maternal singing, and one crossover of live singing: *The Ocean Disc and the Gato Box*. Sound intensity ranged from 50-70 dB. The intervention

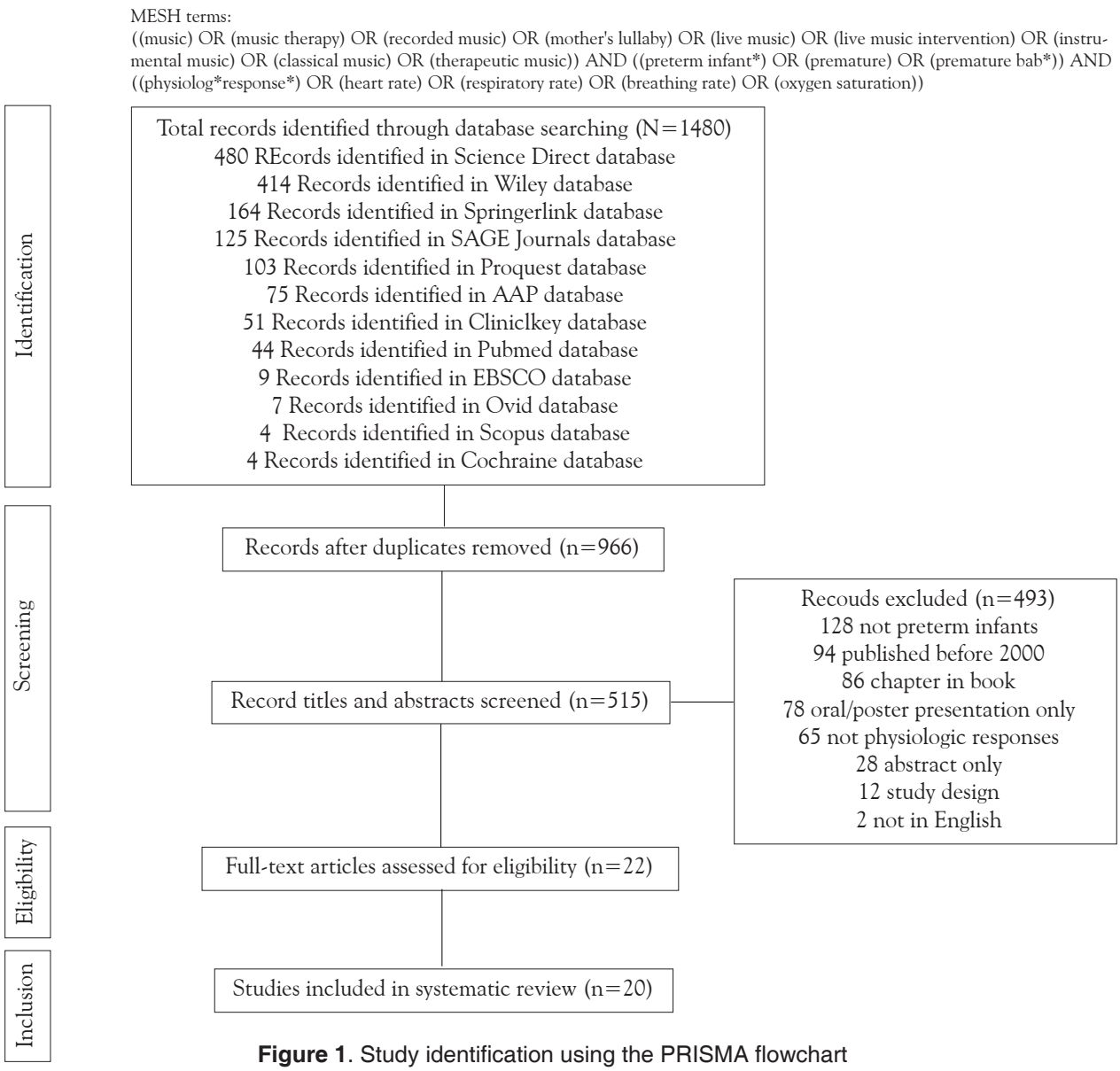

Figure 1. Study identification using the PRISMA flowchart

Table 2. Results of music therapy for preterm infants

Authors(years)	Subjects	Intervention(s) and comparator	Measures	Results
Butt (2000)	14 PI, GA 29–36 weeks	IG: Lullabies music CG: No intervention	HR, SaO_2, behavioral state of arousal (Brazelton), pain response (NFCS)	Significant for all parameters
Calabro (2003)	22 PI, GA 34 weeks, with chronic lung disorder	IG: Lullabies music CG: No intervention	HR, RR, SaO_2, PBAF	NS for all parameters
Arnon (2006)	PI, GA ≥ 32 weeks, BW ≥ 1500 g	IG: (1)Live music and (2)recorded music CG: No music therapy	HR, SaO_2, RR, behavior	HR decreased significantly
Lai (2006)	30 PI (GA ≤ 37 weeks) with BW 1500 g	IG: Lullaby music during KC CG: No intervention	HR, RR, SaO_2, behavioral state, maternal anxiety	NS for all parameters except maternal anxiety state
Johnston (2007)	20 PI, GA 32–36 weeks	IG: Recorded maternal voice CG: No acoustic stimulation	HR, SaO_2	NS
Whipple (2008)	60 PI (GA 32–37 weeks) with BW < 2500 g	IG: Pacifier–activated lullaby (PAL) CG: Pacifier only, no contact	HR, RR, SaO_2	Significant only in RR; HR and SaO_2 NS
Cassidy (2009)	63 PI, GA 28–33 weeks	IG: Recorded lullaby music followed by classic music and same music in reverse order CG: Standard NICU care	HR, RR, SaO_2, head circumference	NS
Farhat (2010)	44 VLBW PI, GA ≤ 34 weeks	IG: Lullaby music CG: No music	HR, RR, SaO_2, weight gain	RR and SaO_2 significantly reduced; HR: NS
Schlez (2011)	52 stable PI, GA 32–37 weeks	IG: KC + live harp music therapy CG: KC only	HR, RR, SaO_2	NS
Amini (2012)	25 stable PI (GA: 28–36 weeks, BW: 1000–2500 g)	IG: Lullaby music and classical music CG: No music	HR, RR, SaO_2	Significant in HR and RR reduction for all intervention; NS for SaO_2
Alipour (2013)	90 PI, GA 28–37 weeks, Apgar min. 7, appropriate weight for GA	IG: Lullaby music headphones with music played and silence headphones CG: No intervention	HR, SaO_2, RR, behavioral state	NS for all parameters
Auto (2013)	61 hospitalized PI (31 IG, 30 CG), GA ≥ 32 weeks, at least 10 days of life	IG: Music therapy with multimodal stimulation with background music CG: Only multimodal stimulation	Body weight gain, HR, RR	Significant reduction in HR and RR
Loewy (2013)	272 PI (GA 32–37 weeks) with RDS, clinical sepsis, SGA	IG: Live music (Lullaby, ocean disc, Gato Box) CG: No intervention	HR, RR, SaO_2, activity level	Significant reduction in HR after all intervention
Arnon (2014)	86 PI, GA 32–36 weeks	IG: KC + live maternal singing CG: KC	HR, RR, SaO_2, behavioral state, STAI Score	NS for all parameters except maternal anxiety
Aydin (2014)	60 PI GA < 37 weeks (30 IG, 30 CG)	IG: classical Turkish music + standard care CG: standard care	Peaked HR, SaO_2, RR, LOS	NS for all parameters
Dearn (2014)	22 PI, GA > 28 weeks	IG: Brahms' lullaby CG: standard NICU care	HR	NS
Jabraelli (2016)	66 PI, GA 29–34 weeks	Brahms' and maternal lullaby CG: Standard NICU care	SaO_2	Significantly increased SaO_2
Taheri (2016)	52 PI, < 37 weeks	IG: recorded male lullaby CG: no music	HR, SaO_2	Significant reduction HR and SaO_2
Wirth (2016)	61 PI (IG Lullabies: 20; IG Maternal Voice 20; CG 21). GA 30–36 weeks	IG: (1) Lullabies and (2) maternal voice CG: Standard care	HR, RR, activity	Significant decreased HR and RR
Caparros-Gonzalez (2018)	17 PI (IG: 9; CG: 8), GA 32–36 weeks	IG: Relaxing tune Melomics computer system CG: Silence	HR, RR, SaO_2, systolic BP, diastolic BP	HR and RR significant

BW=birth weight; CG=control group(s); HR=heart rate; IG=intervention group(s); GA=gestational age; KC=kangaroo care; LOS=length of stay; NIPS=neonatal infant pain scale; NS=not significant; SaO_2=oxygen saturation; PI=preterm infants; PBAF=*Physiological and Behavioral Assessment Form*; REE=resting energy expenditure; RR=respiratory rate; $TcPaO_2$=transcutaneous arterial O_2 pressure; VLBW=very low birth weight

was applied 3 days/week up to once daily for 20–30 minutes, and given for 1-3 days. Music therapy was given 30-60 minutes after feeding,[14-16] either in the morning or afternoon.[17]

Sixteen studies used recorded lullabies with or without vocals, or classical music such as those of Brahms, Sandman, and Mozart. In two studies, the music was delivered using headphones,[11,12] eleven studies used speakers or MP3 players inside or close to the incubator; one study used PAL,[13] two studies did not clearly state the delivery mode used.[18, 19] The decibel levels were set between 45-76 dB. The intervention was offered 1-3 times daily for 10-60 minutes, and given for 1 to 8 days. Music therapy began 30-60 minutes after feeding, nursing, as well as during or after heel stick procedures, at 9-10 AM, 10 AM - 7 PM, 2-3 PM, 8-9 PM, 9-10 PM, or either morning or afternoon.[11-14, 18-27]

Three studies used recorded maternal voice reading a book or singing a song. The recordings were delivered using a tape player or speaker placed inside the incubator, except for one study that did not explain the music delivery method.[19] Sound intensity was set within the range of 55-70 dB. The intervention was given for 10-15 minutes, 1-3 times daily, for 2, 3, or 14 consecutive days. Timing of intervention was after feedings,[28] between 10 AM - 7 PM,[19] and 8-9 PM.[26]

The level of evidence demonstrated by all included studies was level 1b (individual randomized controlled trial). Variations in trial durations showing beneficial effects led to one level of evidence downgrading, although all outcomes were important (**Table 3**). The effects observed in one day[13,25] or two days[16,28] may have influenced potential effects a few days later. Furthermore, the difference of length and timing of intervention also contributed indirectly to quality assessment of the included studies.

Risk of bias analysis of the included studies showed 4 high risk, 14 with some concerns, and 4 low risk (**Table 4**). Participants in 18 studies were explicitly using randomization for grouping. However, only 8 studies reported their randomization methods. Assessors were blinded to group allocation in thirteen studies, not blinded in one study, and the methods for the rest of the studies were not described. Two studies clearly stated a double-blind trial, however, most of the other studies gave no information regarding blinding participants.

Discussion

Music therapy was shown to significantly decrease the heart rate[12,14,17,18,20,23,26,27] and respiratory rate,[13, 18,22,23,26,27] and increase oxygen saturation.[12,19, 20,22] Participants in all studies started from 28 weeks' gestational age. According to fetal auditory development, myelination occurs at the 27th week of gestation, from the cochlea to the auditory thalamus,[29] and external auditory input begins to reorganize the auditory cortex.[30] Music is a complex sound that stimulates multiple sites in the brain, especially the superior temporal lobe. The right hemisphere is primarily responsible for processing some musical components, but a few of these, including perceptual analysis and emotional response,[31] are processed in the other hemisphere.

Neonates are sensitive to temporal stimulus parameters (sound duration) and to higher-order temporal structure (repetition of sound patterns).[32]

Table 3. GRADE quality assessment

Certainty assessment							No. of patients		Effect		Certainty	Importance
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	imprecision	Other considerations	Music therapy	Control	Relative (95%CI)	Absolute (95%CI)		
Heart rate 19	randomized trials	not serious	not serious	serious	not serious	none	811/1064 (76.2%)	223/1064 (21.0%)	not pooled	not pooled	MODERATE	IMPORTANT
Respiratory rate 15	randomized trials	not serious	not serious	serious	not serious	none	739/956 (77.3%)	187/956 (19.6%)	not pooled	not pooled	MODERATE	IMPORTANT
Oxygen saturation 16	randomized trials	not serious	not serious	serious	not serious	none	749/961 (77.9%)	182/961 (18.9%)	not pooled	not pooled	MODERATE	IMPORTANT

Table 4. Revised Cochrane risk of bias tool for randomized trials (RoB 2.0)

		Randomization process				Deviations from intended interventions					Missing outcome data			Outcome measuremenmt		Seleciton bias	
Author (year)	Design	Random sequence generation	Allocation concealment	Baseline imbalances	Equal proportion	Analysis of period effects	Blinding participants	Blinding carers and trial personnel	Deviation from intended interventions	Carry over effects dissapeared	Analyzed in different group	Complete outcome data	Similar proportions and reasons of missing outcome	Evidence of robustness of the missing outcome data	Not blinding assessors	Invluenced by knowledge of intervention	Selective measurement analysis	Overall bias
Butt (2000)	C	NI (some concerns)		No	Yes	NPS	NI (some concerns)	NI	No	NI	NA	Yes (low)	NA	NA	NI (some concerns)	NI	PN (low)	High
Calabro (2003)	P	Yes (low)	Yes	No	NA	NA	NI	No	No	NA	No	Yes (low)	NA	NA	No (low)	NA	No	Low
Arnon (2006)	C	Yes (some concerns)	NI	No	NI	NPS	NI (some concerns)	NI	No	NI	NA	Yes (low)	NA	NA	No (low)	NA	No	Some concerns
Lai (2006)	P	Yes (low)	Yes	No	NA	NA	NI (low)	NI	No	NA	No	Yes (low)	NA	NA	No (low)	NA	No (low)	Low
Johnston (2007)	C	Yes (low)	Yes	No	Yes	NPS	NI (low)	NI	No	Yes	NA	Yes (low)	NA	NA	NI (some concerns)	NI	No (low)	Some concerns
Whipple (2008)	P	Yes (some concerns)	NI	No	NA	NA	NI (low)	No	No	NA	No	Yes (low)	NA	NA	No (some concerns)	NI	No (low)	Some concerns
Cassidy (2009)	P	NI (some concerns)	NI	No	NA	NA	NI (low)	No	No	NA	No	Yes (low)	NA	NA	No (some concerns)	NI	PN (low)	Some concerns
Farhat (2010)	C	Yes (some concerns)	NI	No	NA	NA	NI (low)	NI	No	NA	No	Yes (low)	NA	NA	NI (some concerns)	Ni	No (low)	Some concerns
Schlez (2011)	C	Yes (some concerns)	NI	No	NI	NPS	NI (low)	NI	No	Yes	NA	Yes (low)	NA	NA	NI (some concerns)	NI	No (low)	Some concerns
Amini (2012)	C	Yes (some concerns)	NI	No	Yes	NPS	NI (some concerns)	NI	No	NI	NA	Yes (low)	NA	NA	No (some concerns)	NI	No (low)	High
Alipour (2013)	P	Yes (some concerns)	NI	No	NA	NA	Yes (low)	NI	No	NA	No	Yes (low)	NA	NA	No (low)	NA	No (low)	Some concerns
Auto (2013)	P	Yes (low)	Yes	No	NA	NA	NI (low)	NI	No	NA	No	Yes (low)	NA	NA	NI (some concerns)	NI	No (low)	Some concerns
Loewy (2013)	C	Yes (low)	Yes	No	Yes	NPS	Yes (some concerns)	NI	No	NI	NA	Yes (low)	NA	NA	NI (some concerns)	NI	No (low)	Some concerns
Arnon (2014)	C	Yes (some concerns)	Yes	No	NI	NPS	NI (some concerns)	NI	No	NI	NA	Yes (low)	NA	NA	No (low)	NA	No (low)	Some concerns
Aydin (2014)	P	Yes (some concerns)	NI	No	NA	NA	NI (low)	No	No	NA	No	Yes (low)	NA	NA	NI (some concerns)	NI	No (low)	Some concerns
Dearn (2014)	P	Yes (some concerns)	NI	No	NA	NA	NI (low)	No	No	NA	No	Yes (low)	NA	NA	No (some concerns)	NI	No (low)	Some concerns
Jabraeili (2016)	P	Yes (low)	Yes	No	NA	NA	NI (low)	Yes	No	NA	No	Yes (low)	NA	NA	No	NA	No (low)	Low
Taheri (2016)	P	Yes (low)	Yes	No	NA	NA	Yes (low)	Yes	No	NA	No	Yes (low)	NA	NA	No (low)	NA	No (low)	Low
Wirth (2016)	P	Yes (some concerns)	NI	No	NA	NA	No (low)	No	No	NA	No	Yes (low)	NA	NA	Yes (some concerns)	NI	No (low)	Some concerns
Caparros Gonzales (2017)	P	Yes (some concerns)	NI	No	NA	NA	Yes (low)	Yes	No	NA	No	Yes (low)	NA	NA	No (low)	NA	No (low)	Some concerns

Note: C=crossover randomized trial; NA=pot applicable; NI=No information; NPS=not present significantly; P=parallel randomized trial; PN=probably no; PY=probably yes. *Only applicable in crossover randomized trials. ⁺Only applicable in parallel randomized trials. Overall bias obtained from risk of bias judgement for each bias domains. High risk bias resulted either at least one domain or three or more some concerns. At least one some concerns in each domain makes some concerns in overall bias. Meanwhile, low risk for all domains resulted in low risk of overall bias.

Winkler *et al.*[33] showed that neonates can detect musical beat, as measured by the brain's event-related potentials (ERP). Moreover, Norazadan reported that selective enhancement of neural responses in the brain for beat and meter frequency is induced by music. Different musical rhythms influence brain waves, as measured by electroencephalogram (EEG).[34] Relaxed music induces high power alpha waves and low power beta waves. Alpha waves can influence sympathetic activity.[35] For sounds higher than 100 dB, increased beta power indicates a disturbed state.36 However, the *American Academy of Pediatrics* (AAP) recommends a maximum sound intensity of 65 dB for neonates.[37]

Yamamoto *et al.* found that slow music also decreased norepinephrine levels.[38] In addition, pleasant music increased serotonin and endorphin levels,[39] possibly by reducing cortisol level. Schwilling *et al.* showed a significant decrease in salivary cortisol in very low birth weight infants after music therapy.[40]

Most studies used lullabies or classical music as the intervention. Lullabies have a simple musical structure that infants can clearly differentiate, comprising lower pitch and slower tempo that are used and recognized across cultures.[41] On the other hand, classical music is not soothing, constant, or stable, and is relatively diverse, which may produce alert responses in infants and preterm infants who are still unable to discriminate complicated frames of tunes.[42] However, Amini et al. conducted a study to observe the effects of lullabies and classical music on physiological instability. Both musical types significantly reduced infant heart rate.[23] Among classical music studies, Verrusio *et al.* showed the Mozart effect, in which music increased the alpha band and activity more than Beethoven's music did.[43] Keidar *et al.* found that Mozart's music significantly lowered the resting energy expenditure (REE) for preterm infants to a greater degree than Bach's music.[44]

Most studies that showed significant results in HR, RR, and/or SaO_2 used music intervention between 55-70 dB, lasting for 30 minutes, and given for at least 3 days. We also noted that significant results were obtained in studies that included more mature neonates > 32 weeks. This finding was in line with a trial by Wirth *et al.* they found that preterm neonates with higher gestational age experienced significantly stronger effects on heart rate.[26] Doheny *et al.* also reported that effects of maternal voice on the cardiorespiratory system were observed only in infants ≥33 weeks gestation.[45] Maturation of neurological function occurs during the third trimester of gestation. This might explain the insignificant effects of auditory stimuli on physiological signs in younger infants, since they were not yet able to coordinate the stimuli to an autonomic response.[26] Furthermore, inconsistent results between the Taheri *et al.*[12] and Arnon *et al.*[14] studies might have been related to the differences in duration of intervention, 3 days *vs.* 1 day, respectively. Moreover, subjects' mean gestational ages were 33–34 weeks *vs.* 29 weeks, in the respective studies.[11,16] Arnon *et al.* also stated that preterm neonates at 32 weeks or more who were given live music for 30 minutes showed an improvement in physiological responses.[14] Schlez *et al.* and Lai *et al.* used *Kangaroo Care* (KC) for both treatment and control groups, with no significant effect on HR, RR, or SaO_2, possibly due to stable autonomic activity during KC.[15,21] Skin-to-skin contact leads to multimodal stimulation, including of the tactile-sensory system that develops earlier than the auditory system. Thus, the effect of music might have been masked by the KC intervention.[15]

Although the short-term potential effect of music therapy in preterm infants is still debatable, Schmidt *et al.* showed that, in the long run, musical stimuli increased frontal lobe activity and heart rate (reflecting re-organization and the emergence of emotion) in 9-12 month old infants.[46] As a complementary therapeutic approach, music therapy also offers patient–centered solutions for patient care, comfort, and pleasure, and serves as a low-cost, non-invasive, and easily-implemented method.

Heterogeneity in gestational age of preterm infants, as well as in type, duration, length, frequency, timing of intervention, and outcome measures prevented the authors from performing a meta-analysis. Overall bias in 2 studies was high, and some concerns were raised in 14 studies. Furthermore, larger RCTs are needed to optimize the effects of music therapy.

In conclusion, despite the finding that musical interventions demonstrate promising results in some studies, the variation in quality of the studies, age groups, outcome measures, as well as type and timing of the interventions across the studies, make it difficult

to make a definitive conclusion on the effects of music in preterm NICU infants.

Conflict of Interest

None declared.

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Original Article

Factors associated with pericardial effusion in pediatric systemic lupus erythematosus

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Abstract

Background Cardiovascular involvement in systemic lupus erythematosus (SLE) has been reported to range from 4-78%. Complications can affect all structures of the heart, including the endocardium, myocardium, pericardium, and valves. Pericarditis is the most common manifestation, with an incidence of 11-54% in SLE patients. Pericardial effusion is often observed in patients with pericarditis, and can be confirmed by echocardiography.

Objective To determine factors associated with pericardial effusion in children with SLE.

Methods We conducted a restrospective cross-sectional study by reviewing medical records of children with SLE aged less than 18 years who underwent echocardiography at the Dr. Sardjito Hospital. Yogyakarta, from January 2011 to March 2018. Patients with congenital heart disease or incomplete medical records were excluded. A multivariate logistic regression analysis was done to determine factors that independetly associated with pericardial effusion.

Results Among 165 children with SLE, 73 fulfilled the inclusion criteria. The prevalence of pericardial effusion was 54.8%. Median age was 13 (range 5-17) years and the female-to-male ratio was 8:1. Hemolytic anemia (OR=4.135; 95%CI 1.039 to 16.453; P=0.044) was significantly associated with pericardial effusion.

Conclusion Hemolytic anemia is significantly associated with pericardial effusion in children with SLE. **[Paediatr Indones. 2018;58:227-32; doi: http://dx.doi.org/10.14238/pi58.5.2018.227-32]**.

Keywords: *lupus; children; pericardial effusion; pericarditis*

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disorder in which 20% of patients are diagnosed in childhood. Childhood-onset SLE is associated with higher morbidity and mortality than adult-onset SLE. The number of SLE patients in the Department of Pediatrics, Dr Sardjito Hospital, Yogyakarta has increased at an average of 5-6 patients per year, with 65% survival by the fifth year.[1]

Cardiovascular manifestations of SLE cause high morbidity and mortality.[2] The incidence of pericarditis and pericardial effusion documented by echocardiographic examination varied from 11% to 54%. A much higher incidence of pericardial involvement in SLE was reported in autopsy studies in which histological examination was performed.[3] Nowadays, cardiac manifestations are often mild and asymptomatic. However, they can be frequently recognized by echocardiography and other noninvasive tests. Echocardiography is a sensitive and specific technique in detecting cardiac abnormalities, particularly mild pericarditis, valvular lesions, and myocardial dysfunction.[4]

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In high-income countries, echocardiography is routinely performed for early detection of cardiovascular disorders in SLE, as pericarditis is often asymptomatic.[5] However, in low- and middle income countries including our center, echocardiography has not been a routine examination. In previous studies, the factors associated with pericarditis in SLE were hemolytic anemia, proteinuria, lymphadenopathy, anti-Smith antibodies, Raynaud's syndrome, pleuritis, positive anti-double stranded-deoxyribonucleate (anti-ds-DNA), and low levels of complement-3 (C3). We aimed to determine possible factors associated with pericardial effusion in children with SLE.

Methods

This retrospective study was conducted in the Department of Pediatrics, Dr Sardjito Hospital, Yogyakarta. Data were obtained from patients' medical records. We included children with SLE, based on *American College of Rheumatology* (ACR) 1997 and Systemic Lupus International Collaborating Clinics (SLICC) criteria,[6,7] aged ≤18 years, from January 2011 to March 2018 who underwent echocardiography at the initial diagnosis. In our hospital, we performed echocardiography if there was abnormality that suspected cardiac involvement, it was not a routine procedure. We excluded children with congenital heart disease or incomplete medical records. Among eligible subjects for inclusion, we devided into pericardial effusion and no pericardial effusion group. The minimum required number of subjects was calculated with 0.95 confidence level and 80% power. The study subjects were recruited consecutively.

The subjects' basic characteristics were age at diagnosis, sex, parental education, parental socio-economic, nutritional status, and SLE protocol. The outcomes were classified pericardial effusion and no pericardial effusion based on echocardiography. The independent variables were age at diagnosis, gender, nutritional status, parental education level, parental socio-economic status, and clinical manifestations including proteinuria, hemolytic anemia, and immunological parameters [positive anti-ds-DNA, antinuclear antibodies (ANA) test, and low C3], while the independent variables were the status of pericardial effusion. Pericardial effusion was diagnosed by echocardiography, which was defined as abnormal fluid accumulation in the pericardial space.[3,4,8]

Nutritional status based on WHO 2006 criteria which was defined as abnormal nutritional status, consisted of severe malnutrition (WHZ or BMI for age Z<-3SD), mild to moderate malnutrition (WHZ or BMI for age -3SD<Z<-2SD), overweight/obesity (WHZ or BMI for age Z>+2SD) and normal nutritional status if there was good nutritional status (WHZ or BMI for age -2SD<Z<+2SD). Proteinuria and hemolytic anemia were defined by the ACR 1997 criteria.[7,9-11] Proteinuria was defined as excess protein in the urine, protein excretion higher than 0.5 grams/day or >+3 by dipstick procedure.[9,11] Hemolytic anemia was defined as abnormalities of hemoglobine level based on age and followed by positive Coomb's test.[9-11]

We use some protocols for SLE treatment, consisting of lupus nephritis, severe SLE, and standard SLE protocol. Lupus nephritis protocol was defined as clinical and laboratory manifestations that met ACR criteria consisted of persistent proteinuria >0.5 gram per day or greater than +3 by dipstick, and/or cellular casts including red blood cells (RBCs), hemoglobin, granular, tubular, or mixed.[12] Severe SLE protocol was defined as organ involvement may lead to irreversible damage in the affected organ. For example, gastrointestinal involvement (pancreatitis, vasculitis), lung involvement causing shortness of breath (pulmonary hypertension, pulmonary hemorrhagic), cardiac involvement may develop heart failure (valvular insufficiency, pericardial tamponade), lupus nephritis may develop rapidly progressive renal failure (nephrotic syndrome, kidney failure), CNS disease (psychosis, confusion, disorientation, paresthesias, seizure, cognitive dysfunction, severe unremitting headache, cerebrovascular accident, transient ischemic attack, retinal vasculitis), severe skin involvement (scarring, alopecia, ulcers) and hematology involvement (severe anemia or thrombocytopenia may be life threatening).[13.,14] And standard SLE protocol for patients with SLE who have mild and stable disease (those without major organ involvement and/or comorbidity).[13,14]

Baseline data and outcomes were described using mean, median, or proportion, as appropriate. Chi-square test was used to analyze categorical variables.

Fisher's exact test was used when >20% of the cells in the 2×2 contingency table had a frequency <5. The significance level (α) was 0.05 for two-tailed tests. Multivariate analysis was done by logistic regression test. The ORs and 95%CIs were computed to compare the strength of the association. Statistical analyses were performed with *SPSS software version 20* . This study was approved by the Ethics Committee for Medical Research, Universitas Gadjah Mada Medical School.

Results

Eighty-seven children with SLE underwent echocardiography but 14 had incomplete medical record data (5 without C3 level, 1 without ds-DNA level, 4 without ds-DNA and C3 level, and 4 without ds- DNA/ANA/C3 level). Hence, 73 patients were included in the study, of whom 40 (54.8%) had pericardial effusion (**Figure 1**). The characteristics of subjects are shown in **Table 1**.

Bivariate analysis showed that proteinuria (OR 3.913; 95%CI 1.097 to 13.693; P=0.036) and hemolytic anemia (OR 4.815; 95%CI 1.237 to 18.737; P=0.022) were significantly associated with pericardial effusion. However this study showed that age at diagnosis (pubertal status), sex, anti-ds-DNA,

Table 1. Characteristics of children with SLE

Characteristics	N=73
Median age at diagnosis (range), years	13 (5.0-17.0)
Sex, n(%)	
Male	8 (11)
Female	65 (89)
Nutritional status, n (%)	
Severe malnutrition	7 (9.6)
Mild to moderate malnutrition	12 (16.4)
Good nutritional	51 (69.9)
Overweight	3 (4.1)
SLE protocol, n (%)	
Standard SLE	18 (24.7)
Severe SLE	35 (47.9)
Lupus nephritis	20 (27.4)

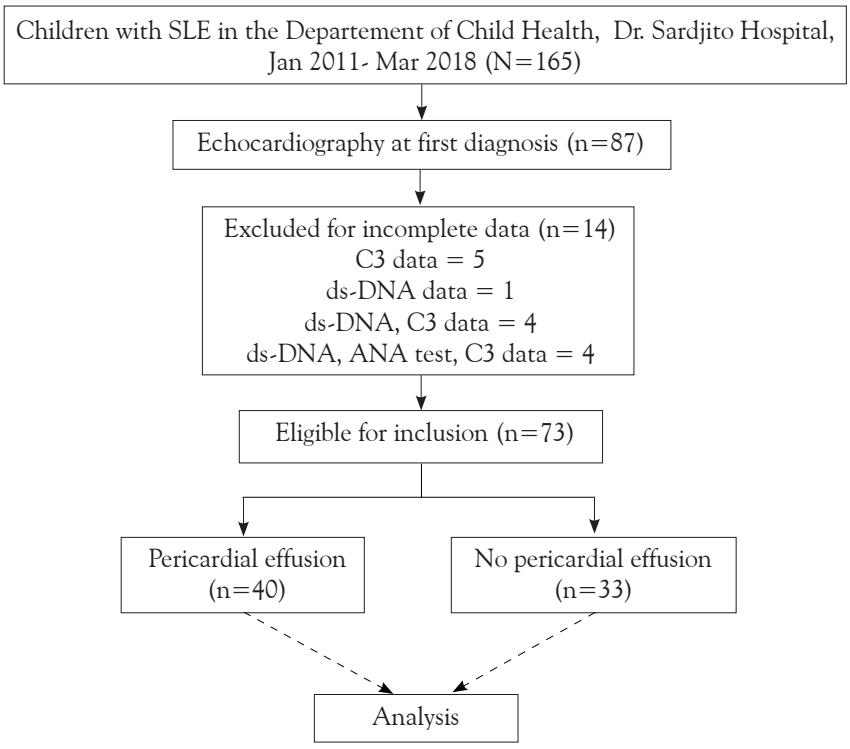

Figure 1. Study flow diagram

ANA test, and C3 at diagnosis were not significantly associated with pericardial effusion (**Table 2**). Multivariate logistic regression analysis showed that hemolytic anemia was an independent factor associated with pericardial effusion (OR 4.135; 95%CI 1.039 to 16.453; P=0.044), but proteinuria was not (OR 3.258; 95%CI 0.885 to 11.998; P=0.076) (**Table 2**).

Discussion

Cardiovascular involvement was prevalent in children with SLE, as about half of our subjects had pericardial effusion. This finding was consistent with previous studies with pericarditis prevalences of around 8-53%.[15-18] Also, characteristics and immunological profiles are similar among children with SLE.[18]

We found that age and biological sex were not associated with pericardial effusion, similar to a previous study in which age and sex were not significantly associated with serositis.[3] Another study noted that pericarditis was not associated with sex, but associated with age at diagnosis.[15] SLE is prevalent during puberty due to the influence of estrogen and androgen hormone inactivity.[1] These hormones affect the pituitary to activate the immune system and cytokine production.[17]

Nutritional status was not associated with pericardial effusion. In contrast, a study conducted in United Kingdom (UK) found that the amount of organ damage was closely related to age at diagnosis, as well as nutritional status.[19] Another study conducted in Brazil, showed that there was relationship between nutritional status and immunity. Malnutrition and obesity can both lead to immunosuppression, which may affect the degree of inflammation already present in SLE.[19,20]

A previous published study in China found no significant differences in laboratory parameters, including proteinuria, in adult-onset SLE patients in terms of pericarditis.[21] We also found that proteinuria was not associated with pericardial effusion

Table 2. Bivariate analysis of possible variables associated with pericardial effusion

Variables	Pericardial effusion (n=40)	No pericardial effusion (n=33)	Bivariate analysis			Multivariate analysis		
			OR	95%CI	P value	OR	95%CI	P value
Pubertal status, n(%)			0.907	0.306 to 2.694	0.861			
Prepubertal	9 (22.5)	8 (24.2)						
Pubertal	31 (77.5)	25 (75.8)						
Sex, n(%)			3.735	0.514 to 14.569	0.280#			
Male	6 (15)	2 (6.1)						
Female	34 (85)	31 (93.9)						
Nutritional status, n(%			0.583	0.213 to 1.598	0.292			
Abnormal	10 (25)	12 (36.4)						
Normal	30 (75)	21 (63.6)						
Clinical manifestation								
Proteinuria, n(%)			3.913	1.097 to 13.963	0.036#	3.258	0.885 to 11.998	0.076
Positive	36 (90)	23 (69.7)						
Negative	4 (10)	10 (30.3)						
Hemolytic anemia, n(%)			4.815	1.237 to 18.737	0.022#	4.135	1.039 to 16.453	0.044
Positive	13 (32.5)	3 (9.1)						
Negative	27 (67.5)	30 (90.9)						
Immunological profile								
Anti-ds-DNA, n(%)			1.280	0.412 to 3.887	0.663			
Positive	32 (80)	25 (75.8)						
Negative	8 (20)	8 (24.2)						
ANA test, n(%)			2.202	0.485 to 10.002	0.306			
Positive	37 (92.5)	28 (84.8)						
Negative	3 (7.5)	5 (15.2)						
C3, n(%)			1.526	0.458 to 5.086	0.492			
Positive	34 (85)	26 (78.8)						
Negative	6 (15)	7 (21.2)						

#Fisher's exact test

on multivariate analysis. However, a study in China found that positive anti-ds-DNA was associated with renal involvement and pericarditis.[22] In addition, another study in South Korea found that proteinuria was associated with pericarditis.[15]

In our study, hemolytic anemia was the only variable significantly associated with pericardial effusion on multivariate analysis, consistent with several previous studies. Another study conducted in United States (US) noted that having hemolytic anemia increased the risk of pericarditis.[10] Moreover, another study in South Korea found that patients with hemolytic anemia had twice the risk of pericarditis.[15] Another study conducted in US observed a strong relationship between hemolytic anemia with IgM or IgG anti-phospholipid (aPL) antibodies. Another study conducted in Italy about heart involvement in SLE, explained that several autoantibodies, such as aPL antibodies, anti- SSA/Ro antibodies, and anti-endothelial cells antibodies, can mediate heart damage.[23] These autoantibodies can directly affect the heart tissue or trigger a mechanism that can cause heart involvement.[15,23,24]

We found that immunological status with regards to anti-ds-DNA, ANA test, and C3 were not associated with pericardial effusion. Consistent with a previous study, positive anti-ds-DNA was not associated with pericarditis.[22] Likewise, another study found no significant differences in laboratory parameters (ANA test, anti-ds-DNA, C3) in patients <15 years of age with regards to pericarditis.[21] In contrast, a previous study noted a significant association between anti-ds-DNA and pericardial effusion by multivariate analysis.[15] A Kuwaiti study in children with SLE found that serologic profiles with positive ANA test, positive anti-ds-DNA, and positive C3 were associated with heart damage.[17] Also, a study conducted in China showed significant associations between positive anti-ds-DNA and C3 with serositis.[25]

This study had several limitations. We used a retrospective design, therefore, risk factors and outcomes were taken from one point of time, and depended on the completeness of medical record data. Also, the small sample size limited the strength of the study. Furthermore, we did not use Kappa test to assess the agreement among examiners who performed echocardiography. However, this study provides preliminary data that may be useful to determine cardiovascular status of SLE patients at the time diagnosis.

In conclusion, pericardial effusion is associated with hemolytic anemia in children with SLE. We suggest performing echocardiography in SLE patients with hemolytic anemia.

Conflict of Interest

None declared.

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Original Article

Immunotherapy and probiotic treatment for allergic rhinitis in children

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Abstract

Background Allergic rhinitis is a global health problem that is increasing in prevalence. Many kinds of therapy have been tried, such as antihistamines, probiotics, and immunotherapy. Immunotherapy may restore the patient's normal immunity against the specific allergen, while probiotics may modify the natural course of allergy.

Objective To evaluate probiotics and immunotherapy for improving clinical symptoms of allergic rhinitis.

Methods This randomized controlled trial (RCT) involved 64 patients, aged 3-18 years, and diagnosed with persistent allergic rhinitis in the Department of Child Health, Sardjito General Hospital from April 2016 until May 2017. Patients were randomly allocated into three therapy groups: group A (standard therapy/cetirizine only), group B (standard and probiotic therapy), and group C (standard therapy and immunotherapy). Clinical symptoms of allergic rhinitis including sneezing, rhinorrhea, and itchy nose, were evaluated for 7 weeks and classified as improved or not improved. The significance of the data was analyzed using proportion test.

Results Sixty-four patients completed 7 weeks of therapy, 15 subjects in group A, 26 in group B, and 23 in group C. Group C showed significantly more improvement of sneezing and rhinorrhea compared to both group A (Z=5.71; Z=7.57, respectively) and group B (Z=2.82; Z=6.90, respectively). However, itchy nose was not significantly improved in group C compared to group B (Z=0.50), but was significantly improved in group C compared to group A (Z=10.91). Group B had significant improvement of sneezing, rhinorrhea, and itchy nose compared to group A (Z=3.81, Z=2.86, and Z=10.91, respectively).

Conclusion The combined standard-immunotherapy group has significantly superior improvement compared to the combined standard-probiotic group and the standard therapy group, in terms of sneezing and rhinorrhea in children with persistent allergic rhinitis. **[Paediatr Indones. 2018;58:280-5; doi: http://dx.doi.org/10.14238/pi58.6.2018.280-5]**.

Keywords*: allergic rhinitis; immunotherapy; probiotics*

Allergic respiratory diseases are major health problems in the pediatric population due to their high prevalence and chronicity, as well as the costs for treatment and effect on quality of life. One of the most important risk factors for the development of airway diseases in children and adolescents is atopy.[1] This condition predominates during the childhood years, with 25% classified as having severe allergic rhinitis.[2] The prevalence of asthma and allergies has increased over the last few decades. Allergic diseases are multifactorial illnesses determined by a complex interplay between genetic and environmental factors.[3] A prevalence and comorbidity study of allergy in children in our previous study on 2014 revealed that 33.8% were diagnosed with allergic rhinitis, 17.3% with atopic dermatitis, and 9.1% with asthma.[4] House dust mites were the most common aeroallergen.[5]

Allergic rhinitis is defined as a type I hypersensitivity allergic reaction with the predominance of Th2 cells and characterized by high IgE levels.[6] The standard therapy for allergic rhinitis is second generation antihistamines, but additional therapy

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may be needed in persistent or severe cases.[7] Probiotic treatment has a unique, disease-modifying effect, as it manipulates the normal flora ecosystem in the gastrointestinal tract, induces the stability of Th1 and Th2 immune responses, and stimulates T-regulators to inhibit excessive Th1 and Th2 reactivity.[8] Probiotic supplementation was shown to be beneficial for decreasing nasal eosinophil percentages in children with allergic rhinitis.[9]

Immunotherapy is given with the aim of modifying the pathogenesis of allergic rhinitis. By giving increasing amounts of allergens to modify the biological response, long-term tolerance may be induced, even after the treatment has ended. This treatment approach has been shown to decrease symptoms and improve quality of life, while being cost-effective for a large number of patients. In addition, it is considered to be the only treatment that can influence the natural course of the disease by targeting the cause of the allergic inflammatory response.[9,10] In allergic rhinitis, the effectiveness of immunotherapy has been demonstrated in many carefully conducted placebo-controlled trials.[11] Skin test sensitivity decreases and allergen-specific IgG increases with immunotherapy.[12] Immunotherapy has also been demonstrated to be quite effective in both seasonal and perennial allergic rhinitis.[10,13] Immunotherapy treatment using house dust mite allergen has been used widely in developed countries for treating allergic rhinitis and asthma, but rarely used in Indonesia.[14,15] The frequency of symptoms has been used as a predictor of immunotherapy effectiveness in asthma patients.[15] Administration of immunotherapy and probiotic adjuvants can improve clinical scores and quality of life in asthmatic children, despite a lack of significant differences in immunological parameters such as IFNγand eosinophils.[17-19] These treatments may increase the CD4+/CD8+ T-cell ratio as well, which may lead to remarkable improvement of clinical symptoms in asthmatic children.[14] This study aimed to evaluate probiotics and immunotherapy for improving clinical symptoms of allergic rhinitis.

Methods

This RCT was conducted from April 2016 to May 2017, on subjects who were diagnosed with persistent allergic rhinitis and treated as outpatients in the Allergy and Immunology Division, Department of Child Health, Dr. Sardjito General Hospital. The inclusion criteria were children aged 3-18 years with persistent allergic rhinitis, at least one positive skin prick test result, and parental written informed consent. Diagnoses were based on the *2016 Allergic Rhinitis and Its Impact on Asthma* (ARIA) classification with symptoms present at least 4 days/week for at least 4 weeks.[21] We excluded those who did not complete 7 weeks of therapy, those with unusual skin prick test results (wide skin lesions or severe dermatographism), antihistamin-dependent,[20] and uncooperative patients.

Subjects were allocated into three groups using a randomized block design and followed up until the 7th week of therapy. The 3 groups were group A (standard therapy/cetirizine only), group B (standard and probiotic therapy), and group C (standard therapy and immunotherapy). We used cetirizine 10mg as standard therapy, a sachet of *Protexin*® for probiotic and a house dust mite allergen with 0.001 concentration form Pharmacy in Dr. Soetomo General Hospital. The improvement of each clinical symptom was evaluated by comparing the frequency of the symptom before and after 7 weeks of therapy. The significance of the data was analyzed using the proportion test. The study protocol was approved by the Medical Research Ethics Committee of the Universitas Gadjah Mada Medical, Public Health, and Nursing School.

Results

A total of 64 subjects aged 3 to 18 years were included in the study and randomly allocated into three groups: 15 in group A, 26 in group B, and 23 in group C. Most subjects were male (10 in group A, 16 in group B, and 16 in group C). The subjects were predominantly between 3 and 12 years of age in group A (12) and group B (20), but group C subjects were mostly >12-18 years of age. Most subjects had a history allergic rhinitis prior to the study, with symptoms of sneezing, rhinorrhea, and itchy nose. The baseline characteristics of subjects are shown in **Table 1**.

The improvement of each clinical symptom was evaluated by comparing the frequency before and after the 7th week of therapy. The comparisons of clinical

Table 1. Baseline characteristics of study subjects

Characteristics	Group A (n=15)	Group B (n=26)	Group C (n=23)
Sex, n			
Male	10	16	16
Female	5	10	7
Age, n			
3-12 years	12	20	10
>12-18 years	3	6	13
Nutritional status, n			
Underweight	0	1	1
Normal	15	21	21
Overweight	0	4	1
History of sneezing, n			
Present	12	24	23
Not present	3	2	0
History of rhinorrhea, n			
Present	10	19	20
Not present	5	7	3
History of itchy nose, n			
Present	8	20	18
Not present	7	6	5
History of atopy, n			
Present	12	22	18
Not present	3	4	5

Table 2. Clinical symptom improvement after 7 weeks of therapy in the standard *vs.* combined standard-probiotic (groups A and B)

Parameter	Standard (A) (n=15)	Standard+probiotic (n=26)	Z score
Sneezing, n			
Improved	9	18	3.81
Not improved	6	8	
Rhinorrhea, n			
Improved	10	19	2.86
Not improved	5	7	
Itchy nose, n			
Improved	7	19	10.91
Not improved	8	7	

symptom improvement between groups are shown in **Tables 2, 3,** and **4**. **Table 2** shows that group B had significantly improved clinical symptoms compared to group A. Sneezing improved in 18/26 of subjects in group B *vs.* 9/15 in group A (Z=3.81). Rhinorrhea improved in 19/26 of subjects in group B *vs.* 10/15 in group A (Z=2.86). Itchy nose improved in 19/26 of group b *vs.* 7/15 in group A (Z=10.91).

Table 3 shows that clinical symptoms of sneezing and rhinorrhea significantly improved in group C compared to group B [sneezing: 17/23 group C *vs.* 18/26 group B (Z=2.82); rhinorrhea: 19/23 group C vs. 19/26 group B (Z=6.90)]. However, while itchy nose symptoms showed vast improvement in both groups B and C, there was no significant difference between groups, with 17/23 of group C *vs.* 19/26 of group B showing improvements (Z=0.50).

Table 4 shows the clinical symptom improvements between groups A and C. Group C had significantly more improvement of clinical symptoms compared to group A [sneezing: 17/23 group C *vs.* 9/15 group A (Z=5.71); rhinorrhea: 19/23 group C

Table 3. Clinical symptom improvement after 7 weeks of therapy in the combined standard-probiotic *vs.* combined standard-immunotherapy (groups B and C)

Parameter	Standard+probiotic (B) (n=26)	Standard+immunotherapy (C) (n=23)	Z score
Sneezing, n			
Improved	18	17	2.82
Not improved	8	6	
Rhinorrhea, n			
Improved	19	19	6.90
Not improved	7	4	
Itchy nose, n			
Improved	19	17	0.50
Not improved	7	6	

Table 4. Clinical symptom improvement after 7 weeks of therapy in the standard *vs.* combined standard-immunotherapy (groups A and C)

Parameter	Standard (A) (n=15)	Standard+immunotherapy (C) (n=23)	Z score
Sneezing, n			
Improved	9	17	5.71
Not improved	6	6	
Rhinorrhea, n			
Improved	10	19	7.57
Not improved	5	4	
Itchy nose, n			
Improved	7	17	10.91
Not improved	8	6	

vs. 10/15 group A (Z=7.57); itchy nose: 17/23 group C *vs*. 7/15 group A (Z=10.91)].

Discussion

Children with persistent allergic rhinitis who received standard (antihistamine) therapy combined with immunotherapy had significantly superior improvement of sneezing and rhinorrhea compared to those who received standard therapy combined with probiotics, and those who received standard therapy alone. Similarly, an RCT by Karakoc-Aydiner *et al.* concluded that house dust mite-sensitized children with asthma and/or rhinitis treated with either subcutaneous injection immunotherapy or sublingual immunotherapy showed better clinical outcome improvements than children who got antihistamine alone.[22] Another study by Smith *et al.* in 2004 also showed significant improvement of reduction in runny nose and sneezing compared between immunotherapy and placebo.[23] Similar study was done as well by Palma-Carlos *et al.* and showed significant improvement of rhinorrhea, sneezing, and conjunctivitis compared with placebo after one year therapy.[24]

Probiotic are beneficial microbes that give benefit to the host, such as normalize the dysbiotic microbiota, which will associate with immunopathology.[9] It is described in a review by Hardy *et al.* in 2013 that probiotic has the ability as immunomodulatory on the cells, molecules and immune response in the gut mucosae.[7]

Subcutaneous injection immunotherapy has been demonstrated to be efficacacious in the management of allergic rhinitis and asthma, even in multi-allergen situations. This therapy has been effective in the prevention of new sensitizations and progression of rhinitis to asthma.[13] Immunotherapy acts on the T helper cells type 1 (Th1/Th2) axis to shift the T cell phenotype away from the allergic Th2 phenotype. More recently, some evidence has emerged to suggest that immunotherapy may promote regulatory T cells action in attenuating allergic symptoms.[10]

From our study, we conclude that immunotherapy combined with antihistamine has better improvement compared with antihistamine only or antihistamine with probiotic.

Conflict of Interest

None declared.

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Original Article

Congenital heart disease in children with Down syndrome in Afghanistan

Abdul Muhib Sharifi, Abdul Rashid Mansoor, Mohammad Akbar Ibrahimi, Abdul Wali, Wali, Khesrow Ekram

Abstract

Background Congenital heart disease (CHD) is frequently cited as the main cause of death in the pediatric Down syndrome (DS) population. The prevalence and spectrum of CHD patterns in DS varies widely worldwide; this variation could be due to sociodemographic, genetic, and/or geographic factors.

Objective To verify the prevalence, pattern, and frequency distribution of CHD in children with Down syndrome.

Methods A three-year retrospective study was conducted in children aged 0-14 years with Down syndrome who underwent echocardiography for possible CHD from January 2014 to December 2016, based on the Pediatric Unit CHD Registry of the Cardiac Research Institute, Kabul Medical University. Clinical, echocardiographic, and outcome data were collected and sorted according to confirmation of the syndrome and echocardiography result.

Results During the three-year study period, 420 DS patients were identified, 286 (68%) of whom had CHDs. The prevalence of isolated and multiple CHD in the 420 children with DS were 38% (160 patients) and 30% (126 patients), respectively. Ventricular septal defect (23%) and atrial septal defect (16.4%) were the most common isolated defects. The combination of VSD and ASD (19.9%) were the most frequent multiple CHDs. The most common associations of CHD were VSD + ASD (19.9%) and VSD + PDA (9%).

Conclusion A high prevalence of CHDs was noted in children with Down syndrome. VSD and ASD are the most commonly diagnosed isolated CHDs in our study. ASD + VSD is the most common multiple CHD pairing. To our knowledge, this is the first extensive study in Afghanistan to demonstrate the pattern and prevalence of CHD associated with Down syndrome. **[Paediatr Indones. 2018;58:312-6; doi: http://dx.doi.org/10.14238/pi58.6.2018.312-6]**.

Keywords: *congenital heart disease; Down syndrome; echocardiography*

Down syndrome (DS) is the most common chromosomal anomaly among children, with a prevalence of 1/700 live births.[1] Congenital heart disease (CHD) undoubtedly affects the progress and survival of children with DS.[2] The worldwide CHD prevalence was estimated to be 6 to 13 per 1,000 live births.[3,4] This prevalence could be higher in Asian countries due to higher rates of consanguineous marriage, diabetes, and obesity.[5-7]

Congenital heart disease (CHD) is the leading cause of mortality and morbidity in the first two years of life in the DS population;1,4 40% to 63.5% of DS patients have CHD.4 The profiles and types of these CHDs may vary in different geographical areas around the world.[7,8] A 2013 study in Norway also suggested seasonal variation of the occurrence of DS and birth defects, providing indirect evidence of the causal role of environmental factors, since genetic factors do not exhibit seasonality.[9]

It is important to be familiar with the prevalence and anatomical characteristics of CHD in DS, as

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well as the associated complications and causes of morbidity and mortality, in order to apply preventative measures and to improve patient quality of life. In addition, because the type of CHD and the timing of repair affect the prognosis, timely treatment of cardiac abnormalities is crucial for optimal survival.[10] The CHDs are the most frequent congenital anomalies in DS cases.[8] The most common CHDs in patients with DS are atrioventricular septal defect (AVSD), patent ductus arteriosus (PDA), atrial septal defect (ASD), ventricular septal defect (VSD), and tetralogy of Fallot (TOF) with AVSD, according to a Norwegian study.[9] The prevalence of CHD in children with DS in Afghanistan has not been well investigated; it could be higher than in Western countries due to higher rates of consanguineous marriage and less access to prenatal care by pregnant women. Despite the high prevalence of CHD in children with DS, little progress has been made in identifying associated factors and causes. There has been a dearth of studies on CHD in children with DS in Afghanistan. Therefore, we aimed to verify the prevalence, pattern, and frequency distribution of congenital heart disease (CHD) in children with Down syndrome at the Cardiac Research Institute of Kabul Medical University.

Methods

A three-year retrospective study was conducted in children aged 0-14 years with Down syndrome, who underwent echocardiography for possible congenital heart disease from January 2014 to December 2016, based on the Pediatric Unit CHD Registry at the Cardiac Research Institute of Kabul Medical University.

The diagnosis of DS was made by local clinicians, based on clinical features and genetic confirmation. Children diagnosed with DS in the region are routinely referred to the Cardiac Research Institute of Kabul Medical University for cardiac assessment. The inclusion criteria comprised all children with DS diagnosis based on typical clinical features and confirmed by cytogenetic studies. The exclusion criteria comprised children with dysmorphic features and not confirmed to be DS by cytogenetic studies. All participants underwent 2-dimensional echocardiographic examination and Doppler studies.

Clinical, echocardiographic, and outcomes data were collected and sorted according to confirmation of the syndrome and echocardiography results. Data analysis was done with *Statistical Package for Social Sciences (SPSS)* software, using simple descriptive statistics such as ratios, proportions, and percentages. Comparison of means was by student's t-test while proportions were compared using the Chi-square test. Statistical significance was defined as $P \leq 0.05$. This study was approved by the Ethics Committee of Pediatric Cardiology Department, Kabul Medical University, Afghanistan.

Results

During the three-year study period, 420 DS patients were identified, of whom 286 (68%) had CHD. The prevalence of isolated and multiple CHDs in 420 children with DS were 38% (160 patients) and 30% (126 patients), respectively. Ventricular septal defect (23%) and atrial septal defect (16.4%) were the most frequently diagnosed isolated defects. VSD + ASD (19.9%) was the most frequent multiple CHD. The most common associations of CHD were VSD + ASD (19.9%) and VSD + PDA (9%) (**Table 1**).

There were no significant differences in CHD frequency between boys and girls (**Table 2**).

There were no significant differences in age and birth weight of children with and without CHD (**Table 3**).

Discussion

This is the first study to address the spectrum of cardiac defects in DS at the Cardiac Research Institute of Kabul Medical University. This study was conducted in the only pediatric cardiac unit in Kabul City, and included patients referred from different hospitals in the whole city, thus providing data on the frequency and pattern of CHD in DS in almost the whole Kabul City. In our study, the overall prevalence of CHD in children with DS was 68%. This rate was slightly higher than other national published studies (Narchi *et al.*[8] 35.2%, Al-Jarallah[10] 49%), and some large population-based studies, such as the California Birth Defects Monitoring Program registry, Torfs CP

Table 1. Prevalence and types of CHDs in children with Down syndrome

Congenital heart diseases	Type of CHD	Number	% of CHD	% of children with DS
No heart disease		134		31.9
Heart disease		286	100	68
Isolated CHD	Total	160	55.9	38
	VSD	68	23	16.1
	ASD	47	16.4	11.1
	AVSD	25	8.7	5.9
	PDA	12	4.1	2.8
	COA	3	1.04	0.71
	PS	5	1.7	1.1
Multiple CHD	Total	126	44.1	30
	VSD+ PDA	26	9.09	6.19
	VSD + ASD	57	19.9	13.5
	VSD + PFO	13	4.5	3.09
	TOF	14	4.8	3.33
	ASD + PDA	16	5.5	3.80

ASD=atrial septal defect, AVSD=atrioventricular septal defect, CHD=congenital heart diseases, COA=coarctation of aorta, PDA=patent ductus arteriosus, PFO=patent foramen ovale, PS=pulmonary stenosis, TOF=tetralogy of Fallot, VSD=ventricular septal defect

Table 2. Gender distribution of CHD status in children with DS

		Male (n=213)	Female (n=207)	Total (N=420)	P value
DS, n(%)	CHD	139 (65.3)	147 (71.0)	286 (68)	0.43
	No CHD	74 (34.7)	60 (29.0)	134 (32)	0.35

Table 3. Age at the time of referral and birth weight of 420 children with DS, by CHD status

	CHD (n=268)	No CHD (n=134)	P value
Mean age (SD), months	15.98 (17.23)	14.8 (16.7)	0.65
Mean birth weight (SD), kg	2.57 (0.46) [N=183*]	2.74 (0.35) [N=84*]	0.13

*Birth weight of some children was not accurately recorded.

(43.9%),[6] Venugopalan P (60%),[18] Salih AF (53%),[22] Vida VL (54%),[23] Ashraf M (50%),[25] Azman BZ (49.3%),[26] Masaki M (50.5%),[27] Amark K (52.5%),[15] and McElhinney DB (65.7%).[17] This variation in the prevalence of CHD in DS can be explained by differing screening programs and diagnostic facilities, as well as the genetic, socioeconomic, and environmental variability of different study populations. Gene-environment interactions and gene-gene interactions may affect certain molecular pathways during embryogenesis. It has been suggested that genetic factors, specific embryological mechanisms, and cell characteristics may determine the pattern of heart anomalies.[23]

The differences of rate of CHD in DS could be due to different genetic, economic, and other aspects of living situations. Afghanistan is a post-war country, with most people living in poverty and having less access to medical facilities during pregnancy, which may affect the prevalence of cardiac malformation. Moreover, the rate of consanguineous marriage is very high in Afghanistan, which may increase the chance of developing cardiac malformations in children. The true prevalence of congenital heart defects in Afghanistan has not been well investigated, and may be higher than in other countries. Further study is needed with a larger sample size to investigate the true prevalence of congenital heart disease among children.

This study shows a high prevalence of CHDs in children with Down syndrome. The VSD and ASD are the most common isolated CHDs in our

subjects, while ASD + VSD is the most common multiple CHD. To our knowledge, this is the first extensive study done in Afghanistan, to demonstrate the pattern and prevalence of CHD associated with Down syndrome.

Conflict of Interest

None declared.

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Original Article

Polymorphisms associated with type 1 diabetes mellitus

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Abstract

Background Type 1 diabetes mellitus (T1DM) is an organ-specific autoimmune disease characterized by T cell-mediated destruction of pancreatic islets. The genetic factors involved consist of at least five vulnerability genes: HLA, INS, CTLA-4, PTPN22, and IL2RA/CD25.

Objective To investigate for associations of PTPN22-1123 G>C SNP and CTLA-4 +49A/G polymorphisms with T1DM.

Methods Case and control groups underwent CTLA-4 +49A/G gene examination from June to December 2017, using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

Results The study population consisted of 30 T1DM patients and 30 healthy subjects with no family history of diabetes or autoimmune diseases. With regards to the PTPN22-1123 G>C SNP, significantly more subjects with T1DM had the GC genotype than the GG genotype (OR 7.64; 95%CI 1.48 to 39.29; P=0.007). For the CTLA-4 +49A/G polymorphism, although the total number of G alleles in the case group was more than that of the control group (OR 2.286; 95%CI 0.804 to 6.945; P=0.118), there were no significant relationships between the frequency of G alleles (P=0.248) and genotypes GG or AG (P=0.293) with the incidence of T1DM. However, the PTPN22-1123 G>C SNP had a significantly positive association with T1DM, and may be considered as a risk factor for T1DM. In contrast, the CTLA-4 +49A/G polymorphism was not recognized as a risk susceptibility factor for T1DM.

Conclusion These study confirms an association between PTPN22-1123 G>C SNP and T1DM, but no significant association between CTLA-4 +49A/G polymorphism and T1DM. **[Paediatr Indones. 2018;58:274-9; doi: http://dx.doi.org/10.14238/pi58.6.2018.274-9]**.

Keywords: *T1DM; PTPN22-1123* G>C; *CTLA-4+49A/G; PCR-RFLP; polymorphism*

Type 1 diabetes mellitus is an autoimmune disease characterized by the infiltration of pancreatic beta cells by immune cells.[1] Beta cells act as regulators of blood sugar levels in the body by producing and secreting insulin when blood sugar levels rise, and maintaining blood sugar levels within physiological limits.[1] Damage may be caused by autoimmune or idiopathic processes. In T1DM patients, insulin secretion is reduced or stalled.[2] In a state without insulin, blood glucose levels rise to higher than normal. The genetic factors involved consist of several vulnerability genes, at least five of which are as follows: HLA, INS, CTLA-4, PTPN22, and IL2RA / CD25.[3]

Bottini *et al.* discovered that tyrosine phosphatase was associated with susceptibility to T1DM, as it is a lymphoid protein encoded by the PTPN22 gene on chromosome 1p13. The PTPN22 gene contributes to susceptibility to T1DM by increasing T-cell regulatory activation.[3] In 2008, Ikegami *et al.*

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sequenced the PTPN22 gene from Japanese and Korean subjects and found five new SNPs in the sample. One of these, the -1123 G>C SNP in the promoter region, was associated with type 1 diabetes in both Japanese and Korean populations.[4] Since genetic markers in disequilibrium are closely related to etiology of disease, polymorphisms can be used to detect relationships. Conventionally, previous studies had shown that certain gene candidates were considered to cause the disease.[4] This approach was used to evaluate the relationship between T1DM and lymphoid-specific phosphatase (LYP) encoded by PTPN22. The LYP belongs to the family of tyrosine phosphatase proteins (PTP) that are involved in preventing spontaneous T-cell activation by dephosphorylating and deactivating T-cells of related receptor kinases and their substrates. The PTPN22 is specifically expressed in lymphocytes and associated with the SH3 domain of the CSK kinase, which suppresses the mediator signaling of T-cell receptors, i.e., Src family kinases (Lck and Fyn). The LYP is one of the most powerful inhibitors of T-cell activation. Recently, missense mutations in the PTPN22 gene were found to be associated with several autoimmune diseases including T1DM, rheumatoid arthritis, and systemic lupus erythematosus. Mutations in this gene were shown to reduce the binding affinity of LYP to CSK, indicating functional relevance for T-cell activation.[3]

There are four polymorphisms in the CTLA-4 gene, one of which is a single nucleotide polymorphism (SNP) in exon 1 (+49 A/G) encoding the co-stimulatory molecules expressed on the surface of activated T-cells.[5,6] The CTLA-4 and CD28 (also located at 2q33) are part of the immunoglobulin superfamily and bind to the B7 molecule on antigen-presenting cells.[6,7] The CTLA-4 has a greater affinity for B7 molecules than CD28, and it downregulates T-cell function. Therefore, CLTA-4 is highly likely to play an important role in T-cell-mediated autoimmunity and susceptibility to autoimmune disease, including T1DM. Several studies confirmed the association between CTLA-4 + 49 A/G and T1DM, in populations in Spain, France, Korea, Italy, America-Mexico, Belgium, Japan, and Iran.[7,8] However, there was a negative correlation in the population of Turkey, Chile, China, England, Egypt, Portugal, and Brazil.[7,8] We aimed to investigate for associations of the PTPN22-1123 G>C SNP and CTLA-4 +49A/G polymorphisms with T1DM.

Methods

This case-control study was done in a South Sumatera population. The case group had 29 children and 1 adult (16 males, 14 females) with T1DM and the control group had 30 healthy subjects (16 males, 14 females) with no family history of T1DM or other autoimmune diseases. Patients were followed up at the Pediatric Endocrinology Outpatient Unit, at Sriwijaya University School of Medicine, Mohammad Hoesin Hospital. All T1DM patients were diagnosed before the age of 15 years and were insulin dependent. Parameters included in the statistical analysis were gender, age at the time they entered the study, severity at onset (presence or absence of ketoacidosis), and family history of T1DM in at least one first-degree relative. The control group consisted of children aged 10-11 years and in the 5th grade of elementary school in South Sumatera. Informed consent was obtained from adult participants and parents or guardians of subjects under 18 years. The study protocol was approved by the Ethics Committee of the Universitas Sriwijaya Medical School.

The PCR-RFLP method involved four stages: (a) genomic isolation of deoxyribonucleic acid (DNA), (b) polymerase chain reaction (PCR), (c) incubation of PCR products with restriction enzymes, and (d) electrophoresis. Three-mL blood specimens were collected from all subjects in EDTA-anticoagulant tubes. PCR was used to identify PTPN22 and CTLA-4 genes inT1DM. PCR reaction specimens contained the following: 0.25 μg genomic DNA specimen in a 25μl PCR reaction containing 50 μM of each dNTP (Boehringer, Germany), 2U of Taq DNA polymerase, 2.5 μL of 10×PCR buffer, and 0.8 μM of each primer. The reaction mixture was first heated at 94°C for 4 min, then amplification was done for 33 cycles in a PCR thermocycler by denaturation at 94°C for 45 s, annealing at 60°C for 45 s and extension at 72°C for 45 sec per cycle. All PCR products were screened and the PTPN22 gene was found in all samples. After PCR, restriction fragment length polymorphism (RFLP) with the Rsa1 enzyme was done. This enzyme serves as an analyzer tool with direct sequencing of

PCR-RFLP for PTPN22. Amplification of the 162 bp genomic region of PTPN-22 genes was performed with forward (5'-CTGATGGTTCCCCACTGTCT-3') and reverse (5'-CTCCACCCCTAAGCACAAAG-3'). Amplification of the CTLA-4 genes was performed with forward (5'-GCTCTACCTCTTGAAGACCT-3'), reverse (5'AGTCTCACTCACCTTTGCAG- 3') primers. The RFLP analysis of CTLA-4 was done using FastDigest BbvI (Fermentas, Germany) in 30 μL total volume by mixing 10 μL of PCR products, 1.0 μL of BbvI restriction enzyme, 2.0 μL 10 X FastDigest green buffer, and 17 μL nuclease-free water. The mixture was incubated at 37 °C for 10 min followed by heating at 65°C for 10 min. DNA fragments were resolved in 2.0% agarose gels. The A allele does not create a restriction site (162 bp), while the G allele creates A restriction site producing two fragments, 88 bp and 74 bp.

The PCR-RFLP analysis of the PTPN22 gene was done using genomic DNA obtained from peripheral blood leukocytes and sequence-specific primers, followed by PCR product digestion with the Rsa1 restriction enzyme. The PCR amplicon was 323 bp. After digestion by Rsa1, we would expect that the wild type allele G (homozygous) would remain undigested (323bp). However, the 323 bp PCR products of heterozygous G and C alleles would be digested by RsaI, yielding fragments of 287 bp and 36 bp (**Figure 1**). In addition, the homozygous C alleles would also yield fragments of the same size as the heterozygous G and C alleles.

The PCR-RFLP analysis of the CTLA-4 gene was done using genomic DNA extracted from peripheral blood of individuals using an MBI Fermentas DNA isolation kit. After PCR amplification, the products (162bp) were digested with the BbvI restriction enzyme. After digestion by BbvI, we would expect that the wild type allele A (homozygous) would remain undigested (162bp). However, the 162bp PCR products of the heterozygous A and G alleles would be digested by BbvI, yielding fragments of 88bp and 74 bp (**Figure 2**). In addition, the homozygous G allele would also yield fragments of the same size as the heterozygous A and G alleles.

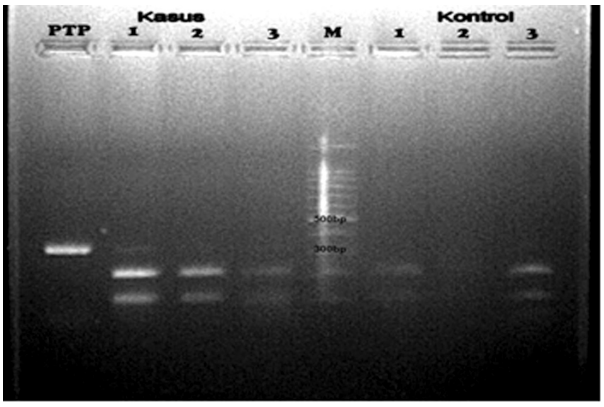

Figure 1. Agarose gel electropherogram of PCR amplicons of the PTPN22 G>C genotype (323bp)

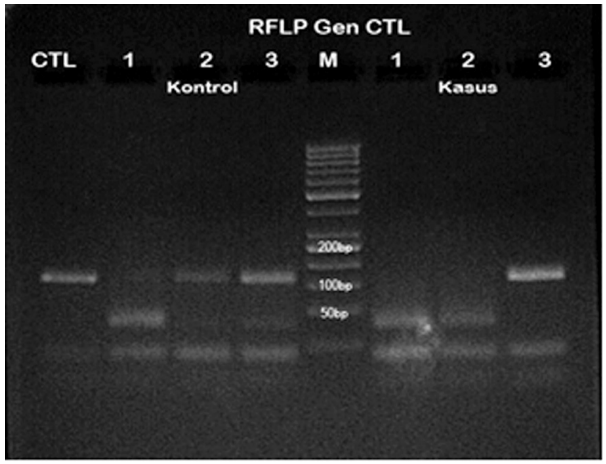

Figure 2. Agarose gel electropherogram of CTLA-4-+49A/G gene PCR amplicon

Data were analyzed using *IBM SPSS Statistics 18* software. The differences in frequencies of genotypes and alleles between case and control groups and among patient subgroups based on qualitative variables, were analyzed by Chi-square or Fisher's exact tests. Normal distribution of quantitative variables was examined using the Kolmogorov-Smirnov test. The level of statistical significance was defined to be $P < 0.05$.

Results

The characteristics of subjects are shown in **Table 1**. Most subjects were male in both groups (16 subjects;). The median age of subjects was 10.5 years, ranging from 3to 29 years. T1DM subjects' nutritional status was mostly well nourished (23 subjects, 77%), while 7/30 subjects were malnourished.

The most common age grouping of T1DM subjects was 6-11 years (15 subjects). All control group

Table 1. Characteristics of subjects

Characteristics	Case (n=30)
Age by group, n	
0-5 years	3
6-11 years	14
12-16 years	8
17-25 years	4
26-35 years	1
Sex, n	
Male	16
Female	14
Nutritional state, n	
Well-nourished	23
Malnourished	7
Race, n	
Austronesia	30
Tribe, n	
Palembang	14
Jawa	1
Minang	1
Komring	1
OKI/OKU/Ogan	4
OI/Kayu Agung	2
Lahat/Gumai	1
Muaraenim/Semendo	2
Banyuasin	2
Sekayu	1
Rawas	1

subjects were aged 6-11 years. All subjects were of the Austronesian race.

One specimen was undetermined, so the total number of specimens was only 59 samples, 30 from the case group and 29 from the control group. With regards to the PTPN22 gene, 43 subjects had a GC genotype. T1DM subjects had significantly more GC genotype and fewer GG genotype (OR 7.647; 95%CI 1.488 to 39.290) than the control group (**Table 2**). Also, the CC genotype was not statistically significant as a risk factor for T1DM (P=0.245). The G allele was significantly more common in the case group than the control group, but presence of the G allele was not a risk factor for T1DM (OR 0.131; 95%CI 0.025 to 0.672; P=0.007). The C allele did not significantly differ between the case and control groups and was also not a risk factor for T1DM. The GG genotype was a significant protective factor against T1DM (OR=0.136; 95%CI 0.27 to 0.690; P=0.02). However, the GC genotype was a significant (P<0.05) risk factor for T1DM (OR=4.53; 95%CI 1.26 to 16.60; P=0.03). The frequency of the allele PTPN22-1123 G>C SNP

Table 2. Correlations between PTPN22 genotypes and alleles with T1DM

Variables	Control (n=29)	Case (n=30)	OR (95%CI)	P value
Genotype, n			7.647	0.007*
GG	10	2	(1.488 to 39.290)¥	
GC	17	26		
CC	2	2		
Allele, n				
G	74	60	0.131 (0.025 to 0.672)	0.007*
C	42	60	0.654 (0.084 to 5.095)£	0.536

* = significant with P<0.05; ¥=OR between groups of GG and GC genotypes; £=Fisher's exact test

was significantly higher in the case group compared to the control group (OR 7.647; 95%CI 1.488 to 39.290; P=0.003).

In the case group, 3/30 subjects had the AA genotype, 11/30 had the AG genotype, and 16/30 had the GG genotype. In the control group, 4/29 subjects had the AA genotype, 16 /29 children had AG, and 10/29 had GG. There was no significant difference in genotype distribution between the case and control groups (P=0.293) as shown in **Table 3**. The A allele was present in 17/30 cases and 24/29 control subjects. The G allele was present in 43 cases (54.4%) and 36 (45.6%) controls. The distribution of A and G alleles was not significantly different among groups (OR 1.68, 95%CI 3.61 to 3.68; P=0.248). For the CTLA-4 +49A/G polymorphism, although the total number of G alleles in the case group was more than that of the control group (OR 2.286; 95%CI 0.804 to 6.945; P=0.118), there were no significant relationships between the frequency of G alleles (P=0.248) and genotypes GG or AG (P=0.293) with the incidence of T1DM.

Table 3. Correlations between CTLA-4 genotypes and alleles with T1DM

Variables	Case (n=30)	Control (n=30)	P value
Genotype, n			
AA	3	4	0.293*
AG	11	16	
GG	16	10	
Allele, n			
A	17	24	0.248
G	43	36	

* = significant with P<0.05

Discussion

Previous studies reported similar T1DM incidences in boys and girls: 2.1 per 100,000 boys and 1.9 per 100,000 girls.[1,3] In our study, the sex distribution was similar in both groups (P= 0.796).

In our study, all T1DM cases were diagnosed at <11 years of age, but the previous study had a mean age <15 years for age at the time of T1DM diagnosis.[9] A limitation of our study was that we could not be sure that the controls did not have T1DM or other autoimmune diseases.

We found a significant relationship between the PTPN22 -1123 G>C gene polymorphism and T1DM. The TIDM cases had a 7.6 times higher chance of having the PTPN22 -1123 G> C polymorphism (P=0.007). The first report of a relationship between the PTPN22 -1123 G> C SNP and T1DM was by Kawasaki et al. in 2006. No other studies have shown that the PTPN22 -1123 G>C SNP could contribute to the tendency of having T1DM, especially in the Asian population. Nonetheless, the researchers concluded that further study was still needed to support this finding.[4]

With regards to CTLA-4, there was no difference in genotype distribution between the two groups (P=0.293). This result was consistent with an Egyptian study which reported no association between the CTLA-4 +49A/G polymorphism and the incidence of T1DM.[10] Similarly, a cohort study in several families in Egypt also found no association between CTLA-4 +49 A/G polymorphism genes and the incidence of T1DM.[11-13] A previous study concluded that the CTLA-4 +49 A/G gene polymorphism was selective for Caucasian race.[13] Studies in Korea, Turkey, Portugal, Chile, and Azerbaijan also found no evidence of a relationship.[7,12] In addition, we found no association of the G allele and genotypes GG and AG with the incidence of T1DM (P=0.248). In contrast, various Middle East studies (Lebanon, Tunisia and Iran) and in Japan showed the relationship between high frequency G allele or GG genotype with the incidence of T1DM.[14,15] Polymorphisms in the CTLA-4 gene have the potential to generate abnormal signals in T helper cells, which may impact the formation of antibodies (by plasma cells) to host cells, including pancreatic β cells, resulting in T1DM.

In conclusion, we confirm the association of PTPN22-1123 G>C SNP with T1DM, but find no significant association between the CTLA-4 +49A/G polymorphism and T1DM. More studies are needed with a larger study population to further confirm these findings.

Conflict of Interest

None declared.

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Original Article

Depression in children with thalassemia major: prevalence and contributing factors

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Abstract

Background Thalassemia major is a chronic disease requiring lifetime treatment. A recent study showed that 11-62% of thalassemia patients developed depression, which is associated with high morbidity and mortality. Understanding the extent of the problem related to depression and its contributing factors is important for early management.

Objective To determine the prevalence and contributing factors for depression in children with thalassemia major.

Methods This cross-sectional study included thalassemia major patients aged 7 to <18 years in the Department of Child Health, Dr. Moh. Hoesin General Hospital (RSMH) in Palembang from June to July 2018 and had received blood transfusions at least 3 times. Subjects completed the Children's Depression Inventory (CDI) questionnaire. Depression was defined as a total score > 13. Data were analyzed using *SPSS for Windows ver. 22.0*.

Results There were 64 patients included in this study, with mean age 12 (SD 3) years and 82.8% female. Most subjects came from families with low socio-economic status and low parental education. Deferiprone was the most commonly used type of iron-chelating agent. Depression was detected in 34.4% of respondents. Multivariate analysis revealed that factors affecting depression in children with thalassemia major were low maternal education (OR 4.014; 95%CI 1.066 to 15.112) and use of deferasirox (OR 4.129; 95%CI 1.168 to 14.601).

Conclusion Prevalence of depression in children with thalassemia major is 34.4%. Low maternal education and deferasirox use as an iron-chelating agent are associated with depression in children with thalassemia major. **[Paediatr Indones. 2018;58:263-8; doi: http://dx.doi.org/10.14238/pi58.6.2018.263-8]**.

Keywords: *depression; children; thalassemia major*

Thalassemia is a common hereditary hematologic disorder. In 2001, the *World Health Organization* (WHO) stated that 7% of people worldwide were carriers for thalassemia and predicted 300,000-400,000 thalassemic newborns yearly.[1-5] The prevalence of thalassemia is still high, particularly in the thalassemic belt region, including Indonesia.[4] According to Dr. Moh. Hoesin General Hospital (RSMH) registry data between June 2010 and April 2018, there were 287 patients with thalassemia, and 145 (50.5%) of them were 7 to <18 years old.[6]

Patients with thalassemia major need lifelong, recurrent blood transfusions and iron-chelating agents, necessitating regular hospital admission and check-ups to increase survival rate.[1-5] Psychosocial problems related to the disease or its treatments may appear in these patients.[4,7-10] One study showed that 80% of patients with thalassemia major easily get psychologic disorders, i.e., depression and anxiety.[10]

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Depression is a chronic mental disorder causing changes in mood, cognitive function, behavior, and physical health. It is a serious illness that can alter the ability to enjoy life and cause decreased functioning capacity, even for simple tasks.[11,12] The mechanism of depression in children with thalassemia can be directly caused by thalassemia, its treatment and complications, or chronic stress. Some factors, like early onset in young patients, recurrent admission for blood transfusions, complications from the disease (anemia and iron overload), and type of iron-chelating agent, are related to the thalassemic patients' psychosocial development and quality of life. Activity limitation, overprotective parents, and school absence can influence social interaction among children and their peers. Other factors that can affect depression in children are age, sex, parental education, family socio-economic status, family history of depression, and poor social support.[7,10-12] Depression in children is related to significant morbidity and mortality because of its recurrence and as a cause of suicide, decreased compliance, drug-use, teen pregnancy, as well as educational and psychosocial function disorders. Early diagnosis and appropriate treatment improves symptoms in 70-80% patients with depression.[7,9,11,12]

Several instruments are useful for screening depression in children, such as the Children's Depression Inventory (CDI).[9,10] Screening is very important so that early management can be implemented before complications arise. To our knowledge, there have been no studies on the prevalence of depression and its contributing factors in children with thalassemia major aged 7-<18 years in RSMH. Our objective was to identify the prevalence of depression in children with thalassemia major and its contributing factors. Identifying such factors can help with prevention, administering early management, preventing complications of depression, and increasing patient quality of life.

Methods

This cross-sectional study was done in the Hemato-Oncology Division, Department of Child Health, RSMH between June and July 2018. Children aged 7 to <18 years with thalassemia major at RSMH and who had been transfused at least three times were consecutively included in this study. Patients with known or ongoing treatment for depression, co-morbid chronic disease like epilepsy, systemic lupus erythematosus (SLE), or leukemia, unable to fill the questionnaire, or not on iron-chelating agent therapy were excluded in this study.

All parents and patients included in this study were given a clear explanation and parents asked to sign an informed consent form before joining the study. Demographic and additional data were recorded from patients' medical records and history-taking from parents. Subjects completed the CDI questionnaire, which comprises 27 questions. Depression was defined as total CDI score of > 13. If positive for depression, subjects were referred to the Department of Psychiatry for further management.

Demographic and clinical data were analyzed descriptively. Factors analyzed for their possible association with depression were gender, age (7-9 years and 10 to <18 years), socio-economic status (low if income per month less than South Sumatra Province minimal wage standard of IDR 2,595,994), parental education (≤ 9 years), family history of depression, frequency of transfusions per month (> 1x per month), type of chelating agent (deferasirox or deferiprone), pretransfusion hemoglobin (< 9 g/dL), ferritin level (≥ 1000 ng/dL), complications of disease (infection, hepatitis, HIV, heart failure, or hormonal disturbances), duration of disease (> 8 years), and duration of iron chelation (> 5 years). Chi-square test and multivariate analysis with binary logistic regression analysis test were performed using *SPSS for Windows version 22.0*. The study protocol was reviewed and approved by the Ethics Committee of RSMH/Sriwijaya University.

Results

There were 64 patients included in this study and most were female (82.8%). Median CDI score was 8, and 22 subjects were deemed to have depression based on their CDI scores. Baseline characteristics of subjects are summarized in **Table 1**.

Children of mothers with low education (<9 years) had 3.4 times higher risk for depression compared to those with mothers with higher

education (P=0.048). The use of deferasirox for iron chelation conferred 1.7 times higher risk for depression compared to deferiprone use (P=0.033). Bivariate analysis results are shown in **Table 2**.

Table 1. Baseline and clinical characteristics of subjects (N=64)

Characteristics	(N=64)
Sex, n (%)	
Male	11 (17.2)
Female	53 (82.8)
Mean age (SD), years	12 (3)
Age by group, n (%)	
7-9 years	16 (25)
10 to < 18 years	48 (75)
Median age at diagnosis (range), years	3 (2-17)
Family socio-economic status, n (%)	
Low	48 (75)
High	16 (25)
Paternal education status, n (%)	
<9 years	37 (57.8)
>9 years	27 (42.2)
Maternal education status, n (%)	
<9 years	42 (65.6)
>9 years	22 (34.4)
Family history of depression, n (%)	
None	62 (96.9)
Positive	2 (3.1)
Frequency of blood transfusions, n (%)	
<1x/month	52 (81.3)
>1x/month	12 (18.7)
Type of iron-chelating agent, n (%)	
Deferiprone	48 (75)
Deferasirox	16 (25)
Mean duration of illness (SD), years	8 (4)
Mean duration of iron-chelating agent use (SD), years	5 (3)
Mean pre-transfusion Hgb level (SD), g/dL	7.18 (0.96)
Hgb level by group, n (%)	
<9 g/dL	63 (98.4)
>9 g/dL	1 (1.6)
Median ferritin level (range), n/dL	3.992 (288-14.000)
Ferritin level by group, n (%)	
<1000 ng/dL	8 (12.5)
>1000 ng/dL	56 (87.5)
Complication of thalassemia, n (%)	
Yes	16 (25)
No	48 (75)
Median CDI score (range)	8 (0-29)
Result of CDI, n (%)	
No depression	42 (65.6)
Depression	22 (34.4)

Multivariate analysis revealed that maternal education <9 years (OR 4.014; 95%CI 1.066 to 15.112) and use of deferasirox as an iron-chelating agent (OR 4.129; 95%CI 1.168 to 14.601) were independent factors positively associated with depression in children with thalassemia major (**Table 3**).

Discussion

Sixty-four subjects in this study had mean age of 12 (SD 3) years and median age at diagnosis of 3 years. These results were similar to those of Sri Rejeki *et al.*, who noted that patients' mean age was 12.28 years and age at diagnosis was 3.78 years.[13] According to CDI scores, 22 subjects (34.4%) had depression. Our result was higher than a Korean study by Shin *et al.*[14] (14%) and a study by Mednick *et al.* (11%).[15]

Various studies have shown that patients with thalassemia major were susceptible for depression.[7,10,15] Depression in patients may be due to the chronicity of thalassemia. Chronic illness may lead reduced confidence, feeling different from their peers, and dependency on others, all of which can leave them feeling isolated and depressed.[10] The differences in proportion of depression in our study may also have been due to genetic and socio-demographic factors, psychological factors, social support, and different type of questionnaires.

Low maternal education status (<9 years) was significantly associated with depression, but paternal education was not. Risk of depression was four times higher in children from mothers with low education status. This result was consistent with a Chinese study, which found that risk of depression in children from mothers with low education was 2.88 times higher compared to children from mothers with higher education. Parents with low educational status may be less sensitive to psychological alterations in their children, thus providing less emotional support to their children. This behavior may dramatically increase risk of depression in children.[16]

In our study, subjects who used deferasirox as an iron-chelating agent had four times higher risk for depression compared to those who used deferiprone. Kontoghiorhers showed adverse neurologic effects in patients using deferasirox. These neurological

Table 2. Factors potentially associated with depression in thalassemia major patients

Factors	Depression, n(%)	No depression, n(%)	OR (95% CI)	P value
Sex				
Male	6 (54.5)	5 (45.5)	2.775 (0.738 to 10.428)	0.117*
Female	16 (30.2)	37 (69.8)		
Age				
7-9 years	7 (43.8)	9 (56.3)	1.711 (0.536 to 5.464)	0.362
10- <18 years	15 (31.3)	33 (68.8)		
Family socio-economic status				
Low	17 (35.4)	31 (64.6)	1.206 (0.359 to 4.501)	0.761
High	5 (31.3)	11 (68.6)		
Paternal education status				
≤9 years	16 (43.2)	21 (56.8)	2.667 (0.873 to 8.413)	0.08
>9 years	6 (22.2)	21 (77.8)		
Maternal education status				
≤9 years	18 (42.9)	24 (57.1)	3.375 (0.973 to 11.708)	0.048
>9 years	4 (18.2)	18 (81.8)		
Family history of depression				
Yes	0 (0)	2 (100)	-	0.247*
No	22 (35.4)	40 (64.6)		
Frequency of blood transfusions				
≤1 x/ month	18 (34.6)	34 (65.4)	1.059 (0.280 to 4.001)	0.608*
>1 x/ month	4 (33.1)	8 (66.7)		
Type of iron-chelating agent				
Deferasirox	9 (56.3)	7 (43.8)	1.667 (0.932 to 2.982)	0.033
Deferiprone	13 (27.1)	35 (72.9)		
Pre-transfusion Hgb level				
<9 g/dL	21 (33.3)	42 (66.7)	-	0.344*
≥9 g/dL	1 (100)	0 (0)		
Ferritin level				
<1000 ng/dL	3 (37.5)	5 (62.5)	1.168 (0.252 to 5.420)	0.565*
≥1000 ng/dL	19 (33.9)	37 (66.1)		
Complications of thalassemia				
Yes	16 (33.3)	32 (66.7)	0.833 (0.257 to 2.703)	0.761
No	6 (37.5)	10 (62.5)		
Duration of illness				
>8 years	10 (31.3)	22 (68.6)	0.758 (0.269 to 2.132)	0.599
≤ 8 years	12 (37.5)	20 (62.5)		
Duration of iron- chelating agent use				
>5 years	10 (27.8)	26 (72.2)	0.513 (0.18 to 1.458)	0.208
≤ 5 years	12 (42.9)	16 (57.1)		

*Fisher's exact test

complications included sleep disturbances, depression, Parkinson's, and anxiety.[17] In contrast, Mednick *et al.* in their longitudinal study on 276 subjects (including 41 children) with thallasemia found no correlation between type of iron-chelating agent and depression.[15]

Table 3. Multivariate analysis of factors affecting depression

Factors	OR (95%CI)	P value
Maternal education < 9 years	4.014 (1.066 to 15.112)	0.040
Deferasirox use	4.129 (1.168 to 14.601)	0.028

We found no correlation between frequency of blood transfusions per month, pre-transfusion hemoglobin level, ferritin level, complications of thalassemia, duration of illness, or duration of iron-chelating agent treatment with depression. More frequent blood transfusion per month was not also correlated with depression. Mednick et al. had similar findings.[15] However, other studies demonstrated

correlations between frequency of blood transfusions and depression. More frequent blood transfusions increased school absences and affected the patients' social interaction with their peers. Hence, they felt isolated and were susceptible to depression.[7,10,18]

Mean pre-transfusion hemoglobin level in our study was 7.18 (SD 0.96) g/dL. Saini *et al.* also showed a similar result, with mean hemoglobin level of 8.5 (SD 1.41) g/dL. There was no correlation between hemoglobin level and depression in either study.[19] Other studies demonstrated that poorly controlled anemia in thalassemia resulted in brain hypoxia that may affect the limbic system and depression.[7,10,19-21]

High ferritin level leads to higher risk for depression because iron gets deposited in many organs including the brain. Hemosiderosis in the hypothalamic and pituitary area alters endocrine function, resulting in decreased serotonin level and depression.[22] Increased iron level also induces production of reactive oxygen species (ROS) which, if affecting the limbic system, causes depression.[22,23] In our study, median ferritin level was 3,992 ng/dL (288-14,000 ng/dL), and there was no significant association with depression. This result may have been because a high majority of children had iron overload, thus the influence of ferritin on depression could not be demonstrated. Saini et al. noted ferritin level of 3,832 (SD 1,796) ng/dL in subjects, but found no association between ferritin and depression.[19] However, Aydinok et al. demonstrated an association between ferritin level > 2,500 ng/dL and depression.[21]

Mean duration of illness was 8 (SD 4) years in our study, but we found no association with depression. However, Saini et al. reported a mean duration of illness of 6.91 (SD 3.08) years, with a statistically significant association between duration of illness and depression and behavior disorders.[19] Other studies stated that the longer the duration of illness, the more frequently patients are regularly hospitalized for blood transfusions, leading to higher risk for complications and depression.[7,10,18]

The cross-sectional study design limited our ability to explore causal relationships between depression and other factors. Another limitation of this study was that we did not include all other psychosocial stressors and we did not investigate potential biological factors (e.g., hormone levels, etc.) in this study.

We recommend routine examinations for children with thalassemia major using the CDI questionnaire for early detection and treatment of depression. Further studies are needed to compare CDI results between patients with thalassemia major and normal subjects and to evaluate the effect of defarasirox on depression. A prospective study for risk factors of depression is also needed. In conclusion, low maternal education and deferasirox use have significant associations with depression in children with thalassemia major.

Conflict of interest

None declared.

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Original Article

Impact of malnutrition on febrile neutropenia in children with acute lymphoblastic leukemia during induction phase chemotherapy

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Abstract

Background Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and adolescents. Febrile Neutropenia (FN) is a medical emergency on ALL that often leads to death. Nutrition status assessment on ALL patient is important because malnutrition can reduce the tolerance of chemotherapy, increase incidence of infection and decrease survival rate.

Objectives To assess malnutrition as a risk factor for FN in children with ALL.

Methods This case-control study was performed at Sardjito Hospital, Yogyakarta on patients aged 1 month to 18 years diagnosed with ALL and undergoing induction phase chemotherapy between January 2013 and December 2015. The case and control subjects were children with and without FN, respectively. Febrile neutropenia was confirmed by patients temperature above 38 ° C at one measurement and a peripheral neutrophil count of less than 1,000/mm3. Malnutrition was defined as body weight-for-height was between -2 and <-3 standard deviation. Subjects were included using simple random sampling.

Results Bivariate analysis showed a significant correlation between malnutrition and FN (OR 2.62; 95%CI 1.07 to 6.45; P=0.03). However, there was no inverse correlation between socioeconomic status and FN (OR 1.1; 95%CI 0.42 to 2.41; P=0.83). There was no correlation between nutritional status and duration of FN (P= 0.48).

Conclusion Malnutrition is a risk factor for FN in children with acute lymphoblastic leukemia. **[Paediatr Indones. 2018;58:298-304; doi: http://dx.doi.org/10.14238/pi58.6.2018.298-304].**

Keywords: *febrile neutropenia; childhood acute lymphoblastic leukemia; nutritional status*

The annual incidence rates of malignancy was reported 186.6 per 1 million children aged birth to 19 years. Acute leukemia is a common malignancy in children.[1] Incidence rate of ALL during 2003-2007 ranged from 1.08-2.12 cases per 100,000 children. Incidence was generally higher in America and Oceania, and the lower incidence in Asia and Eastern countries. In the most countries, the incidence rate of childhood ALL was approximately four times that in adults.[2] The estimated average annual incidence rate of childhood ALL was 20.8 cases per 1,000,000 in Yogyakarta.[3]

Febrile neutropenia is a frequent emergency complication, requiring rapid identification and intervention to save and improve quality of life.[4] In India, the incidence of FN during induction phase chemotherapy was 89.2% of all acute leukemia cases,[5] compared to 47% incidence in Thailand.[6] Kandou Hospital in Manado reported an FN incidence of 22% from all leukemia cases.[7]

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Asturias *et al.* showed that hypotension, C-reactive protein, thrombocytopenia, chemotherapy, nutritional status, and leukemia morphology were not risk factors for FN.[8] Tamam *et al.* showed that poor socioeconomic status was a risk factor for FN (OR 4.59; 95%CI 1.078 to 15.08; P=0,032), while nutritional status was not (P=0.382).[9] Also, a study by Alexandre et al. noted that nutritional and inflammatory status (NIS) were significantly associated with the occurrence of FN.[10] In other studies, FN events were significantly influenced by nutritional status,[11] and malnutrition was a risk factor for FN (RR 24.57; P=0.000).[12]

Assessment of nutritional status in patients with malignancy is important because malnutrition can reduce chemotherapy tolerance, increase the incidence of infection, and decrease survival rate.[13] Malnutrition may be associated with immune response disorders such as impaired phagocyte function, cytokine production, antibody secretion and complement system defects.[14] A critical review of the prognostic value of the nutritional status in children with ALL noted that the mortality rate for children with malnutrition was 1.8 times greater than in ALL children with good nutrition (95%CI 1.72 to 1.88; P <0.001).[15] However, in Yogyakarta to date, there is still no published data on the relationship between nutritional status and FN in children with ALL. The aim of the study was to assess malnutrition as a risk factor for FN in children with ALL, and the results may be used by clinicians or researchers as a reference to improve remission rates and overall survival in children with ALL.

Methods

This case-control study was conducted using medical records data from Sardjito General Hospital. Subjects were children (aged 1 month-18 years) with ALL who underwent induction phase chemotherapy from January 2013 to December 2015. The diagnosis of ALL was based on bone marrow examination, those with FN during induction phase were included in the case group. The control group were patients confirmed ALL and had not FN during induction phase. We excluded patients diagnosed with ALL who previously had been treated with chemotherapy or steroids, as well as patients with clinical finding of Down syndrome, heart failure, or patients with incomplete medical record data (weight, height and socioeconomic status).

Nutritional status assessment was based on the 2006 WHO child growth Z-score for weight-for-height (age <5 years) or body mass index for age (≥5 years). Severe malnutrition was defined as Z-score ≤-3 standard deviation (SD), malnutrition as -2<Z<-3 SD and good nutrition as -2<Z<+2 SD.[16,17] Subject selection was done using simple random sampling to reduce bias. We classified parent education as: primary education (graduated from elementary or junior high school), middle (graduated from senior high school), high (graduated from diploma, bachelor or magister). Socioeconomic status was considered as low if total income of parents is ≤ Rp. 1.300.00,00 per month. Based on prognostic factor, patients were grouped into high risk and standar risk. High risk patients were defined as: age <1 year or >10 years, white blood count was more than 50,000/uL, immunophenotyping was T-cell leukemia, had a mediastinal mass at diagnosis and blast number at peripheral blood >1,000/uL after one week of steroid and 1 dose of intrathecal methotrexate. The remaining patients were classified into standard risk group.[18]

Bivariate analysis results with P values of <0.05 were considered to be statistically significant. Multivariate analysis was done, if needed, by stepwise logistic regression. The results were reported as odds ratio (OR) with 95% confidence interval (CI). This study was approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital.

Results

A total of 228 patients were diagnosed with ALL and received induction phase chemotherapy at Sardjito General Hospital from January 2013 to December 2015. Eighty-three patients were excluded. Of the 145 patients who met the inclusion criteria, 75 patients were treated as cases (FN) and 70 patients as the controls (no FN). Based on minimal sampling calculation and to reduced bias, we took 50 patients as cases and 50 patients as controls. We numbered a

queationnaire at the top right, made a small paper, gave a number (75 cases and 70 controls), folded, mixed and with the closed eyes we took 50 time (each for cases and controls). The subjects' characteristics are presented in **Table 1**.

Of 50 subjects with FN, 44 (88%) developed 1 episode of FN during 7 weeks of induction chemotherapy, 5 (10%) subjects developed 2 episodes of recurrence FN and 1 (2%) subject developed 3 episodes of recurrent FN. Two of 50 (4%) subjects died due to FN (**Table 2**).

Table 1. Characteristics of subjects

Characteristics	FN (n=50)	Without FN (n=50)
Median (range) age at ALL diagnosis, months	56 (14-191)	73 (12-208)
Sex, n (%)		
Male	30 (60)	28 (56)
Female	20 (40)	22 (44)
Paternal education, n (%)		
Primary education	23 (46)	28 (56)
Middle education	18 (36)	12 (24)
High education	9 (18)	10 (20)
Maternal education, n (%)		
Primary education	25 (50)	29 (58)
Middle education	18 (36)	9 (18)
High education	7 (14)	12 (24)
Maternal occupational status, n (%)	38 (76)	20 (40)
Working	12 (24)	30 (60)
Not working		
Risk of therapy group, n (%)		
High risk	32 (64)	25 (50)
Standard risk	18 (36)	25 (50)
Nutritional status, n (%)		
Severe malnutrition	3 (6)	6 (12)
Malnutrition	21 (42)	11 (22)
Over nutrition	2 (4)	0 (0)
Good nutrition	24 (48)	33 (66)
Albumin level, n(%)		
Albumin < 3,5 g/dL	16 (32)	8 (16)
Albumin ≥ 3,5 g/dL	34 (68)	42 (84)
Socioeconomic status, n (%)		
Low	21 (42)	20 (40)
High	29 (58)	30 (60)
Median ANC (range), cells/uL	265 (0-1000)	-

Table 2. Frequency of FN and outcome of FN subjects

Parameters	(N=50)
Frequency FN during induction phase, n (%)	
1 time	44 (88)
2 times	5 (10)
≥ 3 times	1 (2)
Outcome, n (%)	
Died	2 (4)
Alived	48 (96)

Bivariate logistic regression analysis on nutritional status was done using good nutrition as standard compared to severe malnutrition, malnutrition, and over malnutrition. Malnutrition was significantly higher in the FN case group than in the control group (P=0.03). However, severe malnutrition showed no significant differences between good nutrition. We could not analyze between patients with over malnutrition and good nutrition because only two patients had FN and no patient without FN. Bivariate analysis revealed that malnutrition had significant relationship with FN occurrence (OR 2.62, 95%CI 1.07 to 6.45; P=0,03) (**Table 3**).

Further bivariate analysis was conducted to identify confounding factors suspected to affect the occurrence of FN. Socioeconomic status was categorized as low or high, but we found no significant differences between the case and control groups (OR 1.1, 95% CI 0.49 to 2.41, P=0.83). We did not proceed to multivariate analysis because only malnutrition had P values <0.25 (**Table 4**).

We also assessed for a relationship between duration of FN and nutritional status. Gamma correlation test revealed no significant relationship between duration of FN with nutritional status (P=0.48) (**Table 5**).

Table 3. Analysis of nutritional status as a risk factor for FN

Nutritional status	FN (n=50)	Without FN (n=50)	OR	95%CI	P value
Severe malnutrition, n(%)	3 (6)	6 (12)	0.68	0.15 to 3.02	0.,62
Malnutrition, n(%)	21 (42)	11 (22)	2.62	1.07 to 6.45	0.03
Over nutrition, n(%)*	2 (4)	0 (0)	-	-	-
Good nutrition, n(%)**	24 (48)	33 (66)	-	-	-

* Over nutrition vs. good nutrition OR, CI and p value could not be calculated

** Good nutrition as the standard reference

Discussion

The main purpose of this study was to determine if poor nutritional status (malnutrition or severe malnutrition) is a risk factor for febrile neutropenia in children with ALL. A previous study showed that malnutrition increased the risk of infection 2-3 times, as well as lengthened hospital stay and duration of induction phase chemotherapy.[20] Another study reported that malnutrition was correlated with FN in children with ALL underwent induction phase chemotherapy.[12] However, a previous study in our center showed no statistically significant relationship between nutritional status and incidence of FN during induction phase chemotherapy.[21] A Guatemala study also showed no association between nutritional status and FN.[8]

Because of the inconsistent results of previous studies, we aimed to further assess malnutrition as a risk factor for the occurrence of FN in children with ALL who underwent induction phase chemotherapy. A differences of our study, compared to Kholisa study,[21] was the division of nutritional status into 4 categories based on the 2006 WHO chart: severe malnutrition, malnutrition, good nutrition and over nutrition.[16] We noted that few newly diagnosed ALL patients had over nutrition or obesity. The body's response to cancer is to produce TNF, IL-1, and IL-6. The TNF suppresses lipoprotein kinase activity that reduces fat reserves.[22,23] The IL-1 and IL-6 break down protein and decrease albumin synthesis.23 Taken together, these conditions lead to malnutrition in children with ALL. Malnutrition impairs immune function, leading to increased incidence of infection, chemotherapy toxicity, poor quality of life, as well as death.[24,25]

We found that malnutrition was a risk factor for FN in children with ALL during induction phase chemotherapy (OR 2.62; 95%CI 1.07 to 6.45; P=0.03) (**Table 3**). However, severe malnutrition was not a significant risk factor for FN because few of our subject were diagnosed with severe malnutrition. The ALL patients with severe malnutrition undergo severe malnutrition management with oral antibiotics of cotrimoxazole (if no sign of infection) for 5 days or empirical, intravenous antibiotics, in case of infections, consisting of ceftazidime and gentamicin. Cotrimoxazole is a combination antibiotic consisting of sulfamethoxazole (bacteriostatic) and trimethoprim (bactericidal). The spectrum of cotrimoxazole can kill the Gram-positive bacteria (Staphylococcus sp. and Streptococcus), Gram-negative bacteria (Enterobacter sp. and Klebsiella sp), anaerobes, and protozoa. Cotrimoxazole mechanism of action inhibits DNA, RNA and protein formation by blocking the folate pathway.[26] Standard risk group of ALL patients who had FN needed prophylactic antibiotic (Level of Evidence 1B).[27] Prophylactic cotrimoxazole was reportedly effective in preventing pneumocystis pneumonia (PCP) in ALL (Level of Evidence 1A)[28] and was associated with decreased mortality caused by PCP.[29,30]

Table 4. Risk factors for FN in children's ALL (logistic regression)

Risk factors	FN (n=50)	Without FN (n=50)	Bivariate analysis		
			OR	95%CI	P value
Malnutrition, n(%)*	21 (42)	11 (22)	2.62	1.07 to 6.45	0.03
Low socioeconomic status, n(%)*	21 (42)	20 (40)	1.1	0.42 to 2.41	0.83

Note: * no multivariate analysis was performed

Table 5. Analysis of duration of FN and nutritional status

Nutritional status, n(%)	FN ≥ 7 days (n=30)	FN < 7 days (n=20)	Correlation coefficient (r)	P value
Severe malnutrition	3 (10)	0 (0)		
Malnutrition	11 (36)	10 (50)		
Over nutrition	1 (4)	1 (5)	0.17	0.48
Good nutrition	15 (50)	9 (45)		

Notes: Cut off point 7 days was used based on length of care FN.[19] Duration of FN was calculated when the patient had first episode of FN

In addition to antibiotics, severe malnutrition management includes supplements such as zinc and other micronutrients. Zinc has a direct immunomodulatory effect and an indirect effect of protecting the epithelium. Zinc supplementation in long-term malnutrition enhances cellular immunity.[31] Zinc also decreases the duration and severity of FN.[32]

Tamam *et al*. found that poor socioeconomic status was a risk factor for FN (OR 4.59; 95%CI 1.078 to 15.08; P=0.032), while we did not.[9] This difference may be due to most of our patients were covered by the National Health Insurance System to finance either hospitalization or polyclinic treatment. However, using monthly parental income data obtained from medical records as an economic indicator, we found no such association. In this study, low economic status was not connected with FN (OR 1.1; 95%CI 0.42 to 2.41; P=0.83). Overall survival was reported elsewhere as higher in children with ALL those with high than with low socioeconomic status.[33,34]

Other results of our study were similar to previous findings: FN was common in the first and second weeks of chemotherapy administration in induction phase and improved within 14-26 days. This event is due to the timing of ALL diagnosis, in which bone marrow was already in a state of FN, and chemotherapy then made conditions worse.[35] None researchers studied between influence nutritional status with first occurrence of FN.

The number of FN occurrence in our subjects who underwent induction phase chemotherapy for 7 weeks were 44 subjects (88%) with one occurrence, 5 subjects with two occurrence and 1 subject with three occurrence. A previous study showed that FN occurred 2-4 times during 6 months of chemotherapy36 and patients with malnutrition experienced 3 times higher incidence of FN.[14]

The risk of death, comorbidities, bacteremia or infectious complications associated with FN were relatively high in ALL patients.[32,37] In our study, 4% of children with FN died during induction phase, which was less than the 11% who died in a pevious study.[38] Asim *et al.* showed that infection was the dominant cause of death (85%).[37]

We also aimed to determine if duration of FN was influenced by nutritional status (**Table 5**). Gamma correlation test revealed no such association (P=0.48). In contrast, Corner *et al.* found that malnutrition had 1.5 times higher risk of longer hospitalization than good nutrition (95%CI 1.0 to 2.3).[39] This difference may have been due to practice guidelines for treating FN patients regardless of their nutritional status. Those with FN were immediately put in isolation, with restrictions on the number of attendants, guards and medical teams, using strict hand-washing and masks procedures before touching the patient, as well as empirical antibiotics (ceftazidime and gentamicin) in accordance with the most common up-to-date research and types of pathogens.[4,40] In Addition, Avilés-Robles et al. found that patients with sepsis had a long FN duration than those without sepsis (95%CI 1.6 to 2.6).[41]

The shortcoming of this study was the retrospective design, which relied heavily on the accuracy of medical records for socioeconomic status data. Further research is needed to analyze the role of antioxidants (selenium and tocopherol) on the occurrence of FN and improvement of nutritional status. Early nutritional status screening and FN management in children with ALL may be to reduce mortality, shorten treatment length and increase survival rate. Despite the initial nutritional status at the time of ALL diagnosis, malignancy patients need high-caloric content than the normal dietary allowance and can be given in smaller portion,[42,43] also weight checks throughout therapy,43 as chemotherapy side effects can lead to weight loss.[42,43] Prevention of FN requires awareness and education of patient's parents and the medical team.

Conflict of Interest

None declared.

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Original Article

Serum creatinine levels to estimate kidney function in small-for-gestational age and appropriate-for-gestational age newborns

Indra Sandinirwan, Aris Primadi, Dany Hilmanto

Abstract

Background The main parameter used to determine renal function in newborns is serum creatinine. Fetal growth restriction during pregnancy can cause the baby to be born small-for-gestational age. Serum creatinine levels in newborns are affected by muscle mass, gestational age, as well as the number of nephrons and kidney development.

Objective To determine the usefulness of serum creatinine levels as an estimate of glomerular filtration rate in small-for-gestational age and appropriate-for-gestational age newborns.

Methods This cross-sectional study was conducted in May-June 2018. The subjects were full term newborn infants consisting of small-for-gestational age and appropriate-for-gestational age groups (16 subjects each), born in Bandung City Regional Public Hospital. Serum creatinine level was tested by the Jaffe method. The estimated glomerular filtration rate was calculated based on serum creatinine, infant height, and a proportionality constant using the original Schwartz method, eGFR = [k * height]/SCr.

Results Of 32 subjects, there were 17 spontaneous deliveries, 14 males, and 18 females. Mean serum creatinine levels in the small-for-gestational age and appropriate-for-gestational age groups were 0.94 (SD 0.36; 95%CI 0.75 to 1.14) mg/dL and 0.69 (SD 0.18; 95%CI 0.60 to 0.79) mg/dL (mean difference 0.25; 95%CI 0.05 to 0.46; P=0.009), respectively. The median estimated glomerular filtration rates (eGFR) in the small-for-gestational age and appropriate-for-gestational age groups were 25.69 mL/min/1.73m^2 and 30.10 mL/min/1.73m^2 (median difference 4.42; 95%CI 2.04 to 15.8; P=0.008), respectively. There was a weak negative correlation between serum creatinine and birth weight (r=–0.344; P=0.027).

Conclusion Serum creatinine levels in small-for-gestational age newborns are significantly higher than in appropriate-for-gestational age newborns. **[Paediatr Indones. 2018;58:305-11; doi: http://dx.doi.org/10.14238/pi58.6.2018.305-11]**.

Keywords: *creatinine; kidney function; small for gestational age*

Acute kidney injury (AKI) in newborns is estimated to be about 0.4–3.5% of hospital admissions and 8% of hospital occurrences, especially for newborns treated in the intensive care unit. In general, newborns with AKI are born prematurely and/or are critically ill.[1] Currently the guidelines used to determine AKI are the RIFLE criteria (risk, injury, failure, loss, and end-stage renal disease).[2] The RIFLE criteria are used to assess the extent to which renal impairment has occurred and monitor the course of the disease, so that renal impairment does not worsen to eventual end-stage renal disease. Serum creatinine level is one of the indicators for assessing renal function both in RIFLE and neonatal-RIFLE (nRIFLE) criteria.[3]

Serum creatinine is an endogenous biological marker used as a parameter to assess renal function through GFR estimates (eGFR). Serum creatinine is thought to be late in detecting a decrease in GFR

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compared to other biological markers such as urinary neutrophil gelatinase associated lipocalin (NGAL) or cystatin-C.[4,5] Serum creatinine levels may increase up to twice as much from normal values, if there has been a 50% decrease in GFR.[6] Serum creatinine levels are strongly influenced by muscle mass, which not only change with age and height, but also with malnutrition due to prolonged hospitalization.[7] Although some limitations exist, serum creatinine is a simple, accurate, and widely used method to estimate kidney function in both the pediatric and newborn populations.[4,8]

Predisposing factors that can increase the incidence of AKI are small-for-gestational age (SGA) as well as prematurity, perinatal asphyxia, congenital heart disease, and sepsis.[9,10] The SGA newborns appear thin because of decreased muscle mass and subcutaneous fat tissue.[11,12] Creatinine is the result of the breakdown of muscle creatine phosphate, and is produced at a fairly constant level by the body depending on muscle mass. The SGA newborns have less muscle mass and higher risk of impaired renal function due to their smaller number of nephrons compared to appropriate-for-gestational age (AGA) newborns.[10]

To our knowledge, there is no normal value of serum creatinine in SGA newborns and normal values of creatinine levels are generally based on gestational age grouping. The relationship between gestational age and renal maturity in newborns may explain why newborns with lower gestational age tend to have higher serum creatinine levels.[13-15] Hence, we aimed to compare serum creatinine levels of SGA and AGA newborns to estimate kidney function.

Methods

This cross-sectional study was conducted in SGA and AGA newborns. The subjects had 37–42 weeks gestational age according to New Ballard Score (NBS), 5th minute APGAR score ≥7, were aged 48-72 hours, and chosen by consecutive sampling. Determination of the minimum required sample size was based on the sample size formula to test the difference of two averages, resulting in 16 subjects per group. Our 32 subjects consisted of 16 SGA and 16 AGA newborns. The diagnosis of SGA was in accordance with the Lubchenco criteria, i.e., if the birth weight was less than the 10th percentile for the infant's gestational age.[11] Exclusion criteria were newborns whose mothers had kidney disease or acute renal impairment, absence of diuresis within 48 hours, respiratory distress syndrome, sepsis or infection, major congenital abnormalities, multiple congenital anomalies, syndromes, or congenital heart disease.

This study was conducted in the Neonatology Inpatient Ward of Bandung City General Hospital during May-June 2018 and was approved by the Medical Research Ethics Committee of Universitas Padjadjaran (UNPAD). Subjects' parents provided written informed consent. Blood specimens of 2-3 mL were drawn from peripheral veins of subjects at 2-3 days of age, then sent to Hasan Sadikin General Hospital, Clinical Pathology Laboratory for serum creatinine testing using the Jaffe method (*Siemens Dimension EXL 200*). The eGFR was calculated based on serum creatinine, infant height, and a proportionality constant using the original Schwartz method, eGFR = [k * height]/SCr. Height was measured in centimeters (cm), serum creatinine was measured in mg/dL, and the constant value (k) for full term infants was 0.45 in the equation.

The data obtained was analyzed using *SPSS® version 24* for *Windows*. The independent variables in this study were SGA and AGA, while the dependent variable was serum creatinine level. Data analysis was done by T-test if the data were normally distributed or Mann-Whitney test if data was not normally distributed. The significance of the test result was determined based on P values <0.05.

Results

General characteristics of study subjects were gestational age, birth weight, body length, age, sex, mode of delivery, APGAR scores, and complications of childbirth, as shown in **Table 1**. The mean birth weight of the SGA group was lower than the AGA group.

As shown in **Table 2**, mean serum creatinine level in the SGA group was significantly higher (0.94 mg/dL) than in the AGA group (0.69 mg/dL) (P=0.009). The median eGFR in the SGA group was significantly lower (25.69 mL/min/1.73 m^2) than in the AGA group (30.10 mL/min/1.73 m^2) (P=0.008).

Table 1. Characteristics of subjects

Characteristics	Total N=32	Group	
		SGA newborns (n=160	AGA newborns (n=16)
Median gestational age, weeks (range)	38 (37–40)	38 (37–38)	38,5 (37–40)
Birth weight, grams			
Median (range)	2470 (2000–3570)	2300 (2000–2400)	3180 (2540–3570)
Mean	2689	2216	3162
Median birth length, cm (range)	47 (44–52)	45 (44–47)	50 (45–52)
Age, n			
2 days	24	13	11
3 days	8	3	5
Sex (n)			
Male	14	6	8
Female	18	10	8
Mode of childbirth (n)			
Spontaneous delivery	17	9	8
Caesarean delivery	15	7	8
Median APGAR (range)			
1 minute	7 (3–8)	7 (4–8)	7 (3–8)
5 minute	9 (7–10)	9 (8–9)	9 (7–10)
Complications of childbirth, (n)			
History of caesarean delivery		0	3
Twin pregnancy		6	0
Premature rupture of membrane		0	1
Cephalopelvic disproportion		0	3
Inadequate uterine contraction		1	0
Breech presentation		0	1

SGA=small-for-gestational age, AGA=appropriate-for-gestational age

Table 2. Serum creatinine levels and eGFR in the SGA and AGA groups

Variables	SGA (n=16)	AGA (n=16)	Mean or median difference (95%CI)	P value
Creatinine levels, mg/dL				
Mean (SD)	0.94 (0.36)	0.69 (0.18)	0.25 (0.05 to 0.46)	0.009[a]
Median (range)	0.82 (0.59 - 1.68)	0.75 (0.30 - 0.97)		
eGFR, mL/min/1.73m^2				
Mean (SD)	24.25 (7.40)	35.72 (14.32)	4.42 (2.04 to 15.87)	0.008[b]
Median (range)	25.69 (12.05 - 34.32)	30.10 (20.87 - 75.00)		

[a]independent T-test; [b]Mann-Whitney test; *significant: P<0.05

Data on creatinine levels are normally distributed so we use T-test analysis from the mean value measured. Conversely, the eGFR data is not normally distributed so we use the Mann-Whitney analysis from the median value measured instead of mean.

Rank Spearman test revealed a significant negative correlation between serum creatinine level and birth weight (r=–0.344; P=0.027), although the relationship was weak based on the correlation coefficient.

Figure 1 describes the data value of creatinine levels in the study subjects. The normality test shows the data are normally distributed so that the mean value of creatinine levels is used. It can be seen here that the mean creatinine levels of SGA infants were higher compared to AGA infants.

Figure 2 describes the data value of eGFR in the research subject. There are values for outliers in the AGA group with high eGFR. The normality test shows that the data is not normally distributed so that

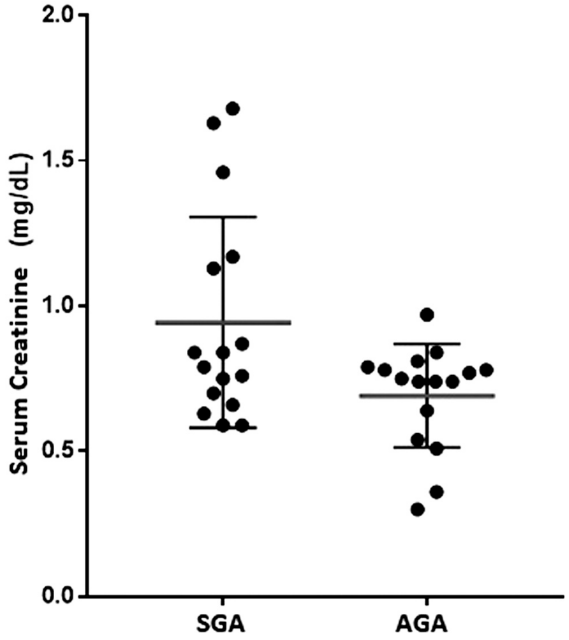

Figure 1. Scatterplot diagram for serum creatinine levels in SGA and AGA infants

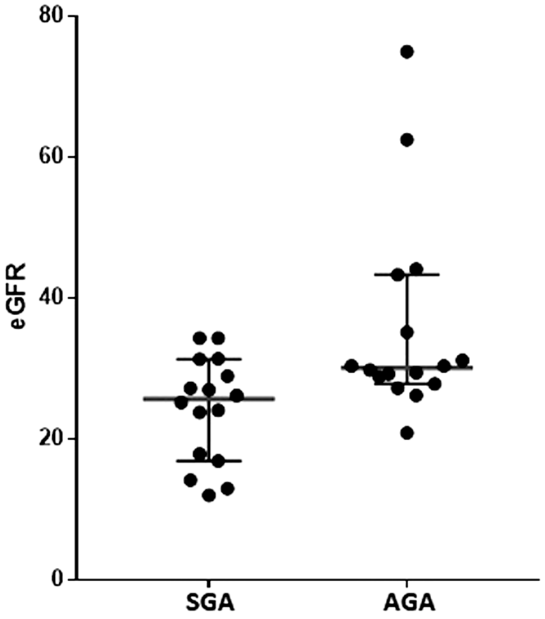

Figure 2. Scatterplot diagram for eGFR value in SGA and AGA infants

the median value of eGFR is used. It can be seen here that the median eGFR value of SGA infants was lower than for AGA infants.

Discussion

Serum creatinine is an endogenous biological marker that is often used to assess renal function via GFR estimation. Creatinine is the product of creatine breakdown. Creatine is synthesized in the liver and present in almost all skeletal muscles in the form of creatine phosphate (CP), an energy storage compound. In the synthesis of ATP (adenosine triphosphate) from ADP (adenosine diphosphate), creatine phosphate is converted into creatine by the catalysis of creatine kinase (CK) enzyme. Along with energy use, a small amount is irreversibly converted to creatinine, which is further filtered by the glomerulus and excreted in the urine.[16,17] Serum creatinine levels in newborns are affected by muscle mass, gestational age, maternal conditions, the number of nephrons, and kidney development. Serum creatinine is thought to be late in detecting a decrease in GFR, and its levels may increase to twice as much as normal if there is a 50% decrease in GFR.[6] Creatinine, in addition to being excreted, is secreted in the renal tubules. Levels increase when capacity of these alternative tubular secretion pathways has been reached. As such, there is a creatinine blind range that limits sensitivity; creatinine levels remain in the normal range during mild GFR decline.[18] Despite some disadvantages, serum creatinine is still an easy and accurate parameter to estimate renal function. Serum creatinine level has been used extensively as an indicator for assessing renal function, including in newborns.[4,19]

In our study, serum creatinine level of SGA newborns was significantly higher (0.94 mg/dL) than the AGA newborns (0.69 mg/dL). Small-for-gestational age infants have birth weight less than the 10th percentile for gestational age, as well as decreased muscle mass and subcutaneous fat tissue caused by intrauterine growth restriction (IUGR).[11] In addition, SGA newborns are at greater risk of impaired renal function due to fewer nephrons compared to AGA newborns.[10]

A previous study reported mean serum creatinine levels in very low birth weight (VLBW), low birth weight (LBW), and normal birth weight infants at 3 days of age to be 0.97 mg/dL, 0.58 mg/dL, and 0.48

mg/dL, respectively. This study also noted mean serum creatinine levels of 3-day-old infants at various gestational ages of 28-32 weeks, 33-37 weeks, and 38-42 weeks with successive results of 1.08 mg/dL, 0.6 mg/dL, and 0.51 mg/dL, respectively.[20] The changes in serum creatinine levels showed relationships between creatinine levels with birth weight and gestational age.[13,17]

An autopsy study reported a lower number of nephrons in SGA infants.[21] Also, another study found significantly lower number of nephrons in SGA infants than in AGA newborns.[22] In addition, Holland *et al.* obtained a linear relationship between the number of glomeruli and birth weight in full term infants, while infants below the 10th percentile for birth weight had 30% fewer glomeruli than infants with birth weight above the 10th percentile.[23] In SGA infants, small kidney weights showed lower number of nephrons, causing the filtration of various proteins including creatinine to be reduced, so that creatinine levels in the circulation increased.[24]

In our study, median eGFR of the SGA group was significantly lower (25.69 mL/min/1.73m^2) compared to the AGA group (30.10 mL/min/1.73m^2) (P=0.008). Heilbron *et al.* reported median GFR in infants at 2-8 days of age to be 39 mL/min/1.73m^2, with a range of 17-60 mL/min/1.73m^2.[25] Marsoosi *et al.* studied the differences of GFR in SGA and AGA infants based on cystatin-C level, and obtained GFRs of 24 (SD 4.4) mL/min in the SGA group and 35.6 (SD 3.2) mL/min in the AGA group.[26] We also noted a similar trend.

The GFR increases as newborns get older; this maturation process of renal function depends on the nephrons already formed. The formation of nephrons stops before the fetus reaches maturation, i.e., at 34-36 weeks gestation.[27] The pattern of kidney growth is centrifugal, with the first nephron formed in the deepest part, i.e., in the juxtamedullary region, and active at birth. The last nephrons formed are in the superficial cortex and undergo further maturation when the juxtamedullary nephron is completed. By the end of pregnancy, the kidneys have approximately 850,000 to 1,000,000 nephrons per kidney. Postnatal maturation continues, with more superficial nephrons attaining proper function. This nephron maturation continues until the age of 18-24 months.[24]

Rank-Spearman test revealed a significant negative correlation between serum creatinine level and birth weight (r=-0.334; P=0.027), with a correlation coefficient indicating a weak relationship. This finding indicates that if the birth weight is low, the serum creatinine level will be higher. Moreover, previous studies support this finding; SGA newborns had fewer nephrons and smaller kidney size based on ultrasound examination.[23,26]

A limitation of our study was potential selection bias when the birth attendant performed the NBS. Newborns who met the inclusion criteria are term newborns based on NBS assessment. According to Ballard *et al.*[28] and Limawal *et al.*,[29] NBS is accurate because it approaches the calculation of the last menstruation period (LMP). In full term infants, the infant's age at the examination does not affect the validity of NBS up to 96 hours of age.[28] However, according to Singhal *et al.*, NBS in SGA newborns overestimated gestational age by 0.7 weeks, especially in the physical maturity aspect.[30]

High serum creatinine in full term newborns even with decreased muscle mass indicates immature kidney function.[31] Given the difference in creatinine levels in SGA and AGA newborns, it is necessary to consider larger-scale studies to determine the creatinine levels that ideally take into account the weight in the SGA and AGA groups. This study provides basic information, so further prospective cohort studies to observe the health outcomes of SGA newborns may also be considered. Considering the kidney immaturity of SGA newborns, this study strengthens the importance of drug dosage adjustment, especially for potentially nephrotoxic drugs.

Conflict of Interest

None declared.

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Original Article

Oxidative stress in neonates with hyperbilirubinemia before and after phototherapy: malondialdehyde and catalase activity

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Abstract

Background Phototherapy is used to treat neonatal hyperbilirubinemia, but is currently thought to cause photodynamic stress and can induce lipid peroxidation. There is increasing evidence that many severe diseases of the neonates are caused by oxidative injury and lipid peroxidation. In the present communique, we review the oxidative succeptibility of the neonates and the evidence now available that phototherapy induces oxidative stress. Malondialdehyde (MDA) is a metabolic product of free radicals. Catalase is an antioxidant that binds free radicals.

Objective To compare the levels of oxidants and antioxidants before and after phototherapy in neonates with hyperbilirubinemia.

Methods This pretest-posttest control group study was conducted in Sanglah Hospital, Bali, from November 2016 to April 2017. Thirty babies with gestational age ≥35 weeks and hyperbilirubinemia with total bilirubin levels requiring phototherapy were included in this study. The MDA levels and catalase activity were measured before and after 24 hours of phototherapy.

Results Comparative analysis using paired T-test showed a significant increase of malondialdehyde level, with mean MDA 23.73 (SD 8.20) nmol/mL before and 53.05 (SD 10.18) nmol/mL after phototherapy ($P<0.001$). However, catalase activity significantly decreased from of 72.33 (SD 10.63) kU/L before phototherapy to 44.85 (SD 14.79) kU/L after phototherapy ($P<0.001$). The MDA level had a significant, negative association with catalase activity after phototherapy ($r = -0.4$; $P=0.028$).

Conclusion Neonates with hyperbilirubinemia are found to have increased oxidative stress after phototherapy, as indicated by increased MDA levels and decreased CAT activity after 24 hours of phototherapy. **[Paediatr Indones. 2018;58:269-73; doi: http://dx.doi.org/10.14238/pi58.6.2018.269-73]**.

Keywords: *hyperbilirubinemia; oxidative stress; malondialdehyde; catalase activity; phototherapy*

Phototherapy is the treatment of choice for hyperbilirubinemia in neonates. It is noninvasive, easy to perform, has few side effects, and is low cost.[1] Some studies have suggested that phototherapy can lead to increased oxidative stress and lipid peroxidation.[2]

Oxidative stress occurs as a result of an imbalance between oxidants, antioxidants, and free radical production. Neonatal red blood cell membranes are more likely to suffer from oxidative stress because it is the dominant pro-oxidants. Neonatal erythrocyte membrane is more susceptible to oxidative damage due to its predominant pro-oxidant potential. The antioxidant activity in serum in term infants is lower compared to that of adults.[3] Oxidative stress can affect lipids, proteins, and DNA, and is believed to play a role in the occurrence of various diseases.[3]

Malondialdehyde (MDA) is a frequently-used marker of oxidative stress and lipid peroxidation in

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vivo.[4] Catalase belongs to the class of hydroperoxidase enzymes that can catalyze the breakdown of hydrogen peroxide (H_2O_2) substrate to water and oxygen, thereby preventing lipid peroxidation of the cell membrane and acting as a free radical binder.[5] The effect of phototherapy on lipid peroxidation remains unclear. One study found a significant increase in MDA levels and parameters of oxidants and a decrease in antioxidant levels after phototherapy.[3] Another study found decreased levels MDA after phototherapy, but increased levels of nitric oxide (NO).[6] Therefore, we aimed to compare malondialdehyde levels and catalase activity in infants with hyperbilirubinemia, before and after phototherapy.

Methods

This pretest-posttest control group design study was conducted from November 2016 to April 2017 in the Neonatal Ward, Sanglah Hospital, Bali. It was approved by the Ethics Review Board at Udayana University, Bali. Subjects were neonates with hyperbilirubinemia requiring phototherapy selected by consecutive sampling. Sample size was calculated based on the two-paired group study formula with type one error of 5% ($Z\alpha=1.96$), type 2 error of 10% ($Z\beta=1.28$), standard deviation of 0.72 and minimum mean differences of 0.45. The minimum required number of subjects was 30 children.

Neonates included in the study had gestational age ≥ 35 weeks, birth weight ≥ 2,500 grams, hyperbilirubinemia requiring phototherapy, and jaundice within days 2-14 of life. Exclusion criteria were neonates with total serum bilirubin (TSB) levels exceeding phototherapy levels and necessitating exchange transfusion, severe asphyxia, major congenital anomalies, or sepsis.

All subjects underwent full history-taking (including medical problems during pregnancy, mode of delivery, delivery events and resuscitation, family history, and postnatal age) and detailed clinical examinations (including birth weight, general examination, as well as chest, heart, abdominal and neurological examinations). Serum bilirubin, MDA, and catalase activity levels were measured at the time of admission (before exposure to phototherapy) and 24 hours after exposure to continuous phototherapy, with the assistance of the clinical pathology laboratory.

Malondialdehyde was measured by a spectrophotometric assay, using the principle that lipid products react with thiobarbituric acid to give a red chromogen, with absorbance at 532 nm. Catalase activity was estimated using an ELISA assay.[2,4,7] Phototherapy was performed for 24 hours, using fluorescent lamps 4 x 20 W as blue light sources, with a wavelength of 420-470 um and light intensity of 10 μW/cm2/nm. The lamp was placed at a distance of 20 cm from the baby's body.

Data was processed with *SPSS 20.0*. Descriptive data were presented in text and tables. Paired student T-test was used to compare bilirubin, MDA, and catalase activity before and after phototherapy. Pearson's correlation test was used to analyze the strength of correlation.

Results

During the study period we enrolled 30 neonates who met the inclusion criteria. Characteristics of subjects are shown in **Table 1**. Two (6.7%) subjects experienced a skin rash and one (3.3%) had hyperthermia during phototherapy. The mean bilirubin level before phototherapy was 18.77 (SD 0.61) mg/dL and after phototherapy was 10.56 (SD 0.72) mg/dL ($P<0.05$). Mean MDA level significantly increased from before [23.73 (SD 8.20) nmol/mL] compared to after phototherapy [53.05 (SD 10.18) nmol/mL] ($P<0.001$). In addition, catalase activity significantly decreased from before [72.33 (SD 10.63) kU/mL] compared to after phototherapy [44.85 (SD 14.79) kU/mL] ($P<0.05$) (**Table 2**). Pearson's correlation

Table 1. Characteristics of subjects

Characteristics	(N=30)
Sex, n	
Male	16
Female	14
Mean birth weight (SD), grams	2955 (325)
Mean age at appearance of jaundice (SD), days	3 (0.5)
Types of delivery, n	
Vaginal	19
Caesarean section	9
Vacuum extraction	2

Table 2. The mean levels of bilirubin, MDA, and catalase activity, before and after phototherapy

Variables	Before phototherapy	After phototherapy	P value*
Mean bilirubin (SD), mg/dL	18.77 (0.61)	10.56 (0.72)	<0.001
Mean MDA (SD), nmol/mL	23.73 (8.20)	53.05 (10.18)	<0.001
Mean CAT activity (SD), kU/L	72.33 (10.63)	44.85 (14.79)	<0.001

*= between before and after phototherapy group (paired T-test)

test revealed a negative correlation between increased MDA levels and catalase activity levels (r=-0.4; P=0.028).

Discussion

We found a significant increase in mean MDA level from before [23.73 (SD 7.33) mmol/mL] to after phototherapy [53.05 (SD 10.18) mmol/mL]. Thiagarajan *et al.* (2014) also noted a significant increase in mean plasma MDA levels after 48 hours of phototherapy [before 12.61 (SD 2.32) μmol/L and after 13.79 (SD 2.85) mmol/L].7,8 In addition, Dahiya et al. reported a significant increase in mean MDA level after 48-96 hours of phototherapy [before 4.62 (SD 0.52) nM/gHb vs. after 5.63 (SD 0.72) nM/gHb] (P<0.001).[1,2,4]

Increased levels of MDA occur due to the formation of free radicals during phototherapy. These free radicals bind to unsaturated fatty acids in the red blood cell membrane, causing lipid peroxidation. Production of free radicals due to phototherapy coupled with low antioxidant defenses in the neonate causes oxidative stress. The reactive oxygen species (ROS) react with lipids, proteins and DNA, producing lipid radicals, alkali-based radicals and sugars, fatty acid radicals, and other radical types. These radical products produce peroxyl radicals when reacting with oxygen, which contribute to oxidative damage. When ROS react with the lipid membrane, the lipid peroxidation results in the formation of lipid hydroperoxide (LOOH), which decomposes into aldehyde forms such as malondialdehyde, 4-hydroxy nonenal (4-HNE) or cyclic endoperoxide, isoprotan, and hydrocarbon forms. Malondialdehyde is the end product of lipid peroxidation processes that can be used as a marker for describing oxidative stress conditions.[2,3]

Abdel Latief *et al.* observed a significant decrease in MDA levels after phototherapy for 12 hours, with 3.28 (SD 0.62) nmol/L before and 2.54 (SD 0.51) nmol/L after phototherapy (P<0.001). This difference to our results may have been due to different phototherapy durations, 12 hours of phototherapy in their study and 24 hours in ours.[6,7,9] The decrease in MDA levels was likely due to improvements in the condition of hyperbilirubinemia and new oxidative stress occurring after greater administration of phototherapy.[8,10]

Several studies have compared oxidative and antioxidant test results before and after phototherapy. We observed a significant decrease of mean CAT activity, with 72.33 (SD 10.63) kU/L before and 44.85 (SD 14.79) kU/L after phototherapy. Dahiya *et al.* also noted that, in addition to increased MDA levels, antioxidant components were significantly decreased after received phototherapy ranging from 48 to 96 hours, such as reduced glutathione (GSH), total thiols, and vitamin C. Supporting results were also obtained by Gulbayzar *et al.* and Block *et al.* Aycicek *et al.*, with significantly decreased antioxidant components such as vitamin C, uric acid, and total antioxidant capacity (TAC), as well as significantly increased total oxidant status (TOS), lipid hydroperoxide, and oxidative stress index after phototherapy for 48 hours (P<0.05).[4,5]

The antioxidant system is classified into two major groups: enzymatic and non-enzymatic antioxidants. A small portion of the oxygen consumed for the aerobic process is converted to an anionic superoxide that must be converted to a less reactive molecule. The major enzymes regulating this process are superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase. The SOD is considered to be first line defense against ROS. This enzyme is present in almost all cells, and converts O_2- into H_2O_2. Mitochondria and SOD bacteria contain Mn, while SOD cytosol is a dimer containing Cu and Zn. The SOD1 and SOD3 contain copper and zinc, whereas SOD2, the mitochondrial enzyme, has manganese in its reactive centre. As H_2O_2 may react with other ROS, it needs to be degraded by one of the other two antioxidant enzymes, GSH-Px or catalase. GSH-Px is located in the mitochondria and

catalyzes the degradation of H_2O_2 by reduction, in which two gluthathione (GSH) molecules are oxidized into glutathione disulfide (GSSG).[7-9]

Pearson's correlation test revealed a significant negative relationship between MDA and CAT activity levels, before and after phototherapy (r=-0.4; P=0.028). Dahiya *et al.* and Thiagarajan *et al.* had similar results in their studies.[3,4,6] Under normal conditions, free radicals produced in the body are neutralized by antioxidants. When free radical levels increase due to phototherapy, the ability of endogenous antioxidants is inadequate to neutralize free radicals, resulting in an unbalanced state between free radicals and antioxidants, which is called oxidative stress. Since MDA is an end product of lipid peroxidation, increased MDA levels and decreased CAT activity are indicative of oxidative stress. Despite the lack of clinical symptoms in our subjects, the damage to lipids, proteins, and DNA occurs at the molecular level. Mutations and damage to DNA may lead to clinical manifestations later in life, especially in those with repeated exposure to conditions causing increased oxidative stress. Such oxidative reactions may also have a role in the occurrence of various allergic diseases (asthma, allergic rhinitis, or conjunctivitis) during childhood.[1,3,4]

A limitation of this study was that while we planned for phototherapy to be done for a full 24 hours, in reality, the implementation of phototherapy was <24 hours, because phototherapy was stopped while mothers breastfed their infants. We did not record the frequency and duration of breastfeeding. One of the management recommendations for hyperbilirubinemia is hydration with breastfeeding on demand. The volume of breast milk given was also not calculated, as it is impossible to do so, hence, study subjects likely received non-standardized volumes of milk.[7,10]

In conclusion, neonates with hyperbilirubinemia are found to have increased oxidative stress after phototherapy, as indicated by increased MDA levels and decreased CAT activity after 24 hours of phototherapy. Therefore, we recommend a very cautious use of phototherapy in all patients with neonatal jaundice.

Conflict of Interest

None declared.

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Original Article

Sepsis calculator to support antibiotic stewardship in early-onset neonatal sepsis: a meta-analysis

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Abstract

Background Establishing a diagnosis of neonatal sepsis is difficult. As such, appropriate timing of antibiotic therapy remains the biggest challenge. As a consequence of non-definitive diagnoses, inappropriate antibiotic administration is common. Recently, a sepsis calculator to estimate risk of early-onset sepsis (EOS) based on both maternal risk factors and infants' clinical presentation was established.

Objective To determine the impact of the sepsis calculator in daily clinical settings, especially with regards to antibiotic usage.

Methods A literature search of Pubmed, EBSCO, Embase, and Scopus database from January 2011 (after sepsis calculator was established) to June 2018 was performed. We included observational studies that compared the sepsis calculator to recent neonatal sepsis guidelines in terms of antibiotic administration, blood culture, and admission to the neonatal intensive care unit (NICU). The literature search, validation study, and assessment risk of bias were done independently by our four authors, while the first author did the statistical analysis.

Results Of the 35 studies identified, 5 cohort studies met the criteria, with a total sample size of 18,352 infants from various countries. We developed a fixed-effect meta analysis of the data. The use of the sepsis calculator significantly reduced inappropriate use of antibiotics [RR 0.46; 95%CI 0.41 to 0.51; $z=13.57$; $P<0.001$], blood culture sampling [RR 0.46; 95%CI 0.40 to 0.52; $z=12.11$; $P<0.001$), and higher neonatal care level admissions [RR 0.68; 95%CI 0.59 to 0.78); $z=5.47$; $P<0.001$). No safety issues were reported from studies using the sepsis calculator.

Conclusion The new EOS risk estimation using a neonatal sepsis calculator is an easy, effective, and safe tool to improve appropriate antibiotic use and outcomes. This calculator is ready to be implemented in all levels of neonatal care units. **[Paediatr Indones. 2018;58:286-97; doi: http://dx.doi.org/10.14238/pi58.6.2018.286-97**].

Keywords: *antibiotic stewardship; early-onset neonatal sepsis; NICU; sepsis calculator*

Early-onset neonatal sepsis (EOS) is an invasive microorganism infection in blood or cerebrospinal fluid in the first 72 hours of life.[1-3] The most common etiologies of EOS are group B streptococcus (GBS), followed by *Escherichia coli*.[1] Early onset neonatal sepsis is usually acquired in the perinatal period shortly before or during birth, due to transplacental, ascending, or intrapartum transmission.[4-6] Early onset neonatal sepsis has one of the highest burdens of neonatal care worldwide.[7-10] With the incidence of culture-proven sepsis ranging from 0.5 to 1.2 cases/1,000 live births, EOS contributes 3 to 40% of mortality in neonatal populations.[11-14] In well-appearing newborns with EOS risk factors, the rate of proven EOS was 0.02 to 0.19%.[15-18]

Following the *Centers for Disease Control and Prevention* (CDC) guidelines for GBS screening and intrapartum antibiotic prophylaxis (IAP), there was a significant decline in both the incidence of overall and GBS-specific neonatal EOS cases.[3,8,15-16] But, these

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guidelines raised physician awareness about antibiotic stewardship, leading to a 200-fold higher antibiotic administration than the incidence of EOS.[19] This phenomenon was contradictory to the antimicrobial stewardship principle endorsed by the *American Academy of Pediatrics* (AAP) since 2007, which consists of maternal antibiotic prophylaxis, antibiotic for neonates suspected to have sepsis (type, duration, rationale), and the approach to the asymptomatic newborn.[20] The critical issue in treatment is rooted in the difficulty to definitively diagnose EOS cases. Clinical presentation of sepsis in neonates is not always indicative of current infection status. Neonates could appear with or without persistent physiologic abnormalities, hemodynamic instability, seizures, and persistent need for supplemental oxygen/mechanical ventilation.[1] Blood culture, along with antibiotic sensitivity test, as the gold standards for sepsis diagnosis and definitive therapy, take time. To date, the *Committee on the Fetus and Newborn AAP* and CDC algorithms do not specify how to interpret the recommended laboratory tests or how to evaluate EOS in terms of duration and severity[3,21-22]

Another consideration in EOS diagnosis is maternal chorioamnionitis (CAM). It is a clinical, traditional, and yet less reliable predictor of upcoming neonatal EOS. Due to wide variations in the diagnostic criteria, no single consensus is has been reached. Regarding clinical signs and symptoms of CAM, they were found in less than 50% of proven EOS cases.[23] The advances in intrapartum antibiotic treatment since the CDC recommendation was implemented, although associated with lower EOS rate in newborns from CAM mothers, have raised the issue of antibiotic stewardship.[10,24-25] However, given the difficulty of diagnosis, it is not surprising that EOS can often be misdiagnosed, and ergo, mistreated. To date, risk stratification based on maternal factors and neonatal clinical findings is still the best approach to assess the possibility of EOS.[26] In 2011, Escobar *et al.* made a breakthrough in perinatal medicine by launching the neonatal sepsis calculator, widely known as the *Kaiser Permanente Neonatal Sepsis Calculator*.[13] The calculator was constructed from a nested case-control study analyzing 350 culture-positive cases and 1,063 matched controls. It is now available on a website and/or mobile-phone based system. The simple and user-friendly calculator is a more efficient approach to measure the probability of EOS in infants born >34 weeks gestation. By entering values from five objective maternal risk factors (of chorioamnionitis) at the time of birth as well as the infant's evolving clinical presentations during the first 12 hours of life, the model results in risk for sepsis per 1,000 live births. The risk is classified into three groups: <0.65 (low risk), 0.65-1.54 (medium risk), and >1.54 (high risk) and the score is classified as "well-appearing," "equivocal," or "clinical illness." This handy EOS risk predictive model helps clinicians to reduce overtreatment by immediately stratifying neonates into 1 of 3 category treatment strategies (continue observation, evaluate with treatment conditional on further information, or treat empirically with antibiotics) efficiently.[11,13,19,27-29] After a validation study in 2016, neonatal centers across the world began to implement this calculator in daily practice.

The aim of this meta-analysis study was to estimate the impact of sepsis calculator usage in routine clinical settings, focusing on antibiotic stewardship. First, we evaluated if inappropriate antibiotic use could be clinically reduced without missing any cases of positive blood culture as the safety issue. Second, we assessed if the sepsis calculator application would minimize over-diagnosis of EOS, thus reducing blood culture sampling and unneeded higher neonatal care level admissions, according to each institution standard.

Methods

We followed the *Preferred Reporting Items for Systematic Reviews and Meta-Analysis* (PRISMA) guideline in constructing this meta-analysis.30 We did a comprehensive electronic literature search from PubMed, EBSCO, EMBASE, and Scopus about studies on associations between the use of the neonatal sepsis risk calculator and outcomes related to antibiotic stewardship, consisting of inappropriate antibiotic use, blood drawn for culture, and higher neonatal level care admission. We used a combination of vocabularies or any possible keywords for early-onset neonatal sepsis, neonatal sepsis calculator, risk stratification, antibiotic use, blood culture, and higher neonatal care admission. The search dates were set from 1 January 2011 to June 2018, with no language restriction. We chose this

time range because the neonatal sepsis calculator was first established in 2011. We also manually searched references from relevant publications to ensure that no publication was missed. We did not seek information from conference proceeding abstracts nor unpublished studies, as these data may not hold up in the peer review process. This literature search was done in June 2018. When a number of publications from the same institution with similar or overlapping patient populations were spotted, only the report published with the largest series was included.

We included any observational cohort studies that compared the sepsis calculator to recent neonatal sepsis guidelines, in terms of either one or more of following outcomes: antibiotic administration, blood culture, and admission to higher level neonatal care. We allowed prospective cohort, retrospective cohort, historical cohort, or any modified cohort with countable relative risk as predetermined effect size for meta-analysis. Exclusion criteria were studies with unclear methods, studies that enrolled neonates <34 weeks of gestation, and studies that included only healthy neonates or neonates without probable infection. We also excluded retrospective chart review or retrospective simulation, reviews, case reports, and non-original studies, such as expert opinions, correspondences, and editorials.

Neonatal sepsis guidelines referred to any guideline used by the neonatal care unit, whether CDC guideline or the institution's own guideline. Early-onset neonatal sepsis was defined by blood or cerebrospinal fluid (CSF) culture with positive results for pathogenic bacteria or fungi, or sepsis in a newborn during the first 72 hours of life. Other common skin pathogens from culture results were regarded as contaminants. Inappropriate antibiotic was defined as the gap between the number of patients given antibiotics compared to the number of patients advised to receive antibiotics from the neonatal sepsis calculator, of those who did not present with clinical deterioration for the first 72 hours of life. We did not assess the type of antibiotic or the duration of antibiotic administration to determine the appropriateness of antibiotic administration, since those values were based on clinical findings of which several considerations could bias the result. Higher neonatal care level admission was defined as the admission of newborns to higher level of neonatal care level compared to each center's policy. For example, several institutions had protocols of care that preterm newborn should be hospitalized in the perinatology unit (Level II), so that higher level was referred to as NICU admission (Level I). Four investigators (HGH, DUN, SMA, and AY) independently evaluated and reviewed the studies found from the literature search. Disagreements, if any, would be resolved by consensus of all authors and by using the Delphi method.

Each author performed individual literature searches, followed by a detailed review of all studies that met our criteria. Details of individual study characteristics included authors, year of publication, study design, sample size, main characteristics of the study population, study outcomes, and study limitations. We extracted data on sample size, relative risk/risk ratio of outcomes, and associated 95% confidence intervals for our statistical analyses.

In cases in which major discrepancies between the data reported in the included studies and the data calculated were observed, or any additional information needed was not reported in the published articles, an electronic-mail was sent to the corresponding authors requesting clarification regarding the raw data of the studied patient group. If we received no reply, such articles were excluded from the meta-analysis. Quality of the studies was assessed with the N*ewcastle-Ottawa Scale* for non-randomized controlled trials and the GRADE system for evidence ranking.[31-32] Any disagreements were resolved by the Delphi method.

The main effect size of this meta-analysis was relative risk. We extracted relative risk values of each outcome and calculated each study weight based on their standard of error. We manually calculated relative risk values for studies that did not implicitly report them, since it was possible that a study only reported the relative reduction of outcomes. We log-transformed each relative risk and upper-lower confidence interval of each study before conducting the meta-analysis. Weighing of each study was done using the inverse-variance method. Results are presented in forest plots. Besides the quantitatively reported data on those outcomes, we also assessed the safety of implementing the neonatal sepsis calculator by finding any missed case identification or fatal outcome in newborns that had low risk of sepsis and no advice on antibiotic administration according to the sepsis calculator.

To investigate any potential presence of publication bias, we chose funnel plot, Begg's rank test, or Egger's regression test as the most suitable methods for bias assessment, according to the literature search result. The heterogeneity of outcomes from studies was expressed by Cochran Q-statistic and inconsistency tests (I2 test). A result was considered to have significant variation if the I2 score was > 20%. We did not conduct subgroup analyses since the neonatal sepsis calculator was aimed for use in the general newborn population. Statistical analysis was done using *STATA 14 software for Windows* and conducted by authors (RR and HGH).

Results

Of the 35 studies, five cohort studies met our predetermined criteria and were eligible for the meta-analysis (**Figure 1**). These studies principally originated from the United States (1 from Arkansas, 1 from Portland, and 1 from Philadelphia), 1 from Australia, and 1 from The Netherlands. All five studies were published between 2016 and 2018 and made use of the neonatal sepsis calculator. Study designs were prospective cohort (1 study) and historical cohort (4 studies).

Study characteristics, outlined in **Table 1**, showed nearly similar patient baseline characteristics. Two studies only included newborns of 35 and 36 weeks gestational age, however, we concluded that these studies were valid for inclusion.[33,36] According to Escobar *et al.*, more than 90% of the study population were newborns with term gestational age.[13] The quality of evidence analysis revealed that all studies had good quality and met the criteria for further data analysis, as shown in **Table 2**.

The outcomes in each study are presented in **Table 3**. We then developed a fixed-effect meta-analysis based on two main reasons: 1) the study populations did not differ much among the studies, and 2) the procedure was reproducible (same

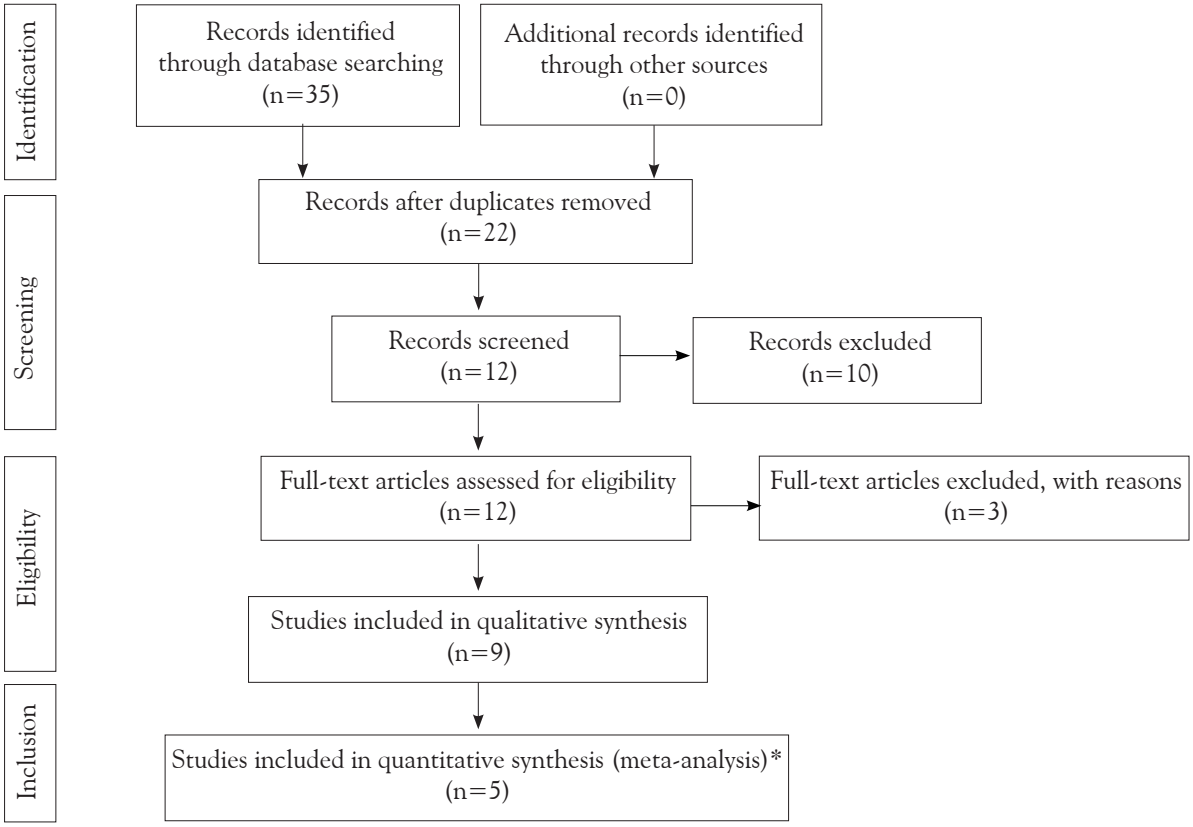

Figure 1. Flowchart of study selection
*reasons for exclusion showed in **Supplementary Appendix 1**

Table 1. Summary of study characteristics

Author (year of publication & place of study)	Study design	Inclusion criteria	Exclusion criteria	Calculator	EOS incidence (per 1,000 live birth)	Outcomes studied	Conclusion
Strunk *et al.*[33] (2018, Australia)	Prospective historical cohort	Infant > 35 weeks	Not clearly stated	Kaiser	0.44	Differences in admission and readmission rates, antibiotic administration, and blood culture sampling	No discrepancy between sepsis calculator and newborn with proven-EOS
Beavers *et al.*[34] (2018, Arkansas-US)	Prospective historical cohort	Infant > 34 weeks, maternal chorioamnionitis	Congenital anomaly	Kaiser	Not clearly stated	NICU admission rate, blood culture drawn, antibiotic administration, total charges, total bed charges, and length of stay	No escalation of care associated with calculator use
Warren *et al.*[35] (2016, Portland-US)	Prospective cohort	Infant > 34 weeks	Antibiotic indication other than EOS	Kaisar	0.5	Comparing antibiotic administration based on CDC guideline and Kaiser calculator, association between I;T ratio > 0.3, and calculator recommendation	No discrepancy between sepsis calculator and newborn with full-course of antibiotics (>5 days)
Dhudasia *et al.*[36] (2018, Philadelphia-US)	Prospective historical cohort	Infant > 36 weeks	Infant underwent CBC testing only	Kaiser	0.49	Proportion of newborns in each risk stratification category, antibiotic use, laboratory testing (blood culture, CBC, CRP, differential count, or combination)	One patient developed clinical deterioration at 36 hours of age, previously categorized as low risk sepsis by calculator (Negative GBS status, no intrapartum antibiotic and no maternal fever)
Achten *et al.*[37] (2018, Netherlands)	Prospective historical cohort	Infant > 35 weeks, elevated maternal EOS risk (based on maternal fever, positive GBS status, rupture of membranes <24 hours before birth, or presumed chorioamnionitis)	Congenital anomaly	Kaiser	0.6	Antibiotic use in each EOS risk category, duration of antibiotics, time to start of treatment, newborn completed 7 days or more of antibiotic treatment, and physician adherence to calculator recommendation	No discrepancy between sepsis calculator and newborns with proven EOS

Table 2. Assessment of study quality using NOS Scale and GRADE system

Author (year of publication)	Quality assessment (NOS) scale				Risk of bias	Final GRADE evidence ranking
	Selection (max. 4)	Comparability (max. 2)	Outcome (max. 3)	Overall score (total 9)		
Strunk *et al.*[33] (2018)	4	2	3	9	Low	Moderate +++
Beavers *et al.*[35] (2018)	4	2	3	9	Low	Moderate +++
Warren *et al.*[35] (2016)	4	1	3	9	Low	Moderate +++
Dhudasia *et al.*[36] (2018)	4	2	3	9	Low	Moderate +++
Achten *et al.*[7] (2018)	4	2	3	9	Low	Moderate +++

Table 3. Results of studies and limitations

Author (year of publication)	Sample size	Culture proven EOS	Results, RR (95%CI)			Safety issue	Study limitation
			Use of AB	Blood culture sampling	Higher neonatal care level admission		
Strunk *et al.*[33] (2018)	4,233	2	0.55 (0.42 to 0.71)	0.67 (0.55 to 0.82)	0.79 (0.67 to 0.92)	No	Low sample size relative to proven EOS incidence
Beavers *et al.*[34] (2018)	255	0	0.39 (0.29 to 0.52)	0.54 (0.43 to 0.68)	0.40 (0.30 to 0.54)	No	Not clearly stated
Warren *et al.*[35] (2016)	202	0	0.25 (0.19 to 0.32)	-	-	No	Not clearly stated
Dhudasia *et al.*[36] (2018)	11,782	4	0.58 (0.50 to 0.69)	0.24 (0.19 to 0.30)	-	No	No assessment of post-discharge infants
Achten *et al.*[37] (2018)	1,877	4	0.46 (0.18 to 0.88)	-	-	No	High missing data rate

neonatal sepsis calculator used and same definition of outcomes), such that any variety among studies was believed to arise from different population sampling only.

From five studies, the use of sepsis calculator reduced inappropriate use of antibiotics [RR 0.46 (95%CI 0.41 to 0.51); P<0.001; z=13.57], as shown in **Figure 2**. Blood culture sampling was also found to be reduced in three studies [RR 0.46 (95%CI 0.40 to 0.52); P<0.001; z=12.11), as was reduced higher level neonatal care admissions [RR 0.68 (95%CI 0.59 to0.78); P<0.001; z=5.47), shown in **Figure 3** and 4, respectively. The lower risk of blood culture was similar to lower antibiotic use. Although significant, less studies investigate on neonatal care admission since several neonatal center have a protocol of neonatal admission based on gestational age, not on clinical condition or current working diagnosis.

We found no reports of safety issues in any studies, as shown in **Table 3**. Although the safety rate of the neonatal sepsis calculator was not implicitly stated, one study reported a newborn with clinical deterioration at 36 hours of age who had been previously stratified as "no need of antibiotic" by the calculator. This infant was eventually admitted to the NICU. All the studies reported that this neonatal sepsis calculator was well implemented in each of their neonatal care centers.

We did not assess publication bias using funnel plot or any advanced regression-based assessment,

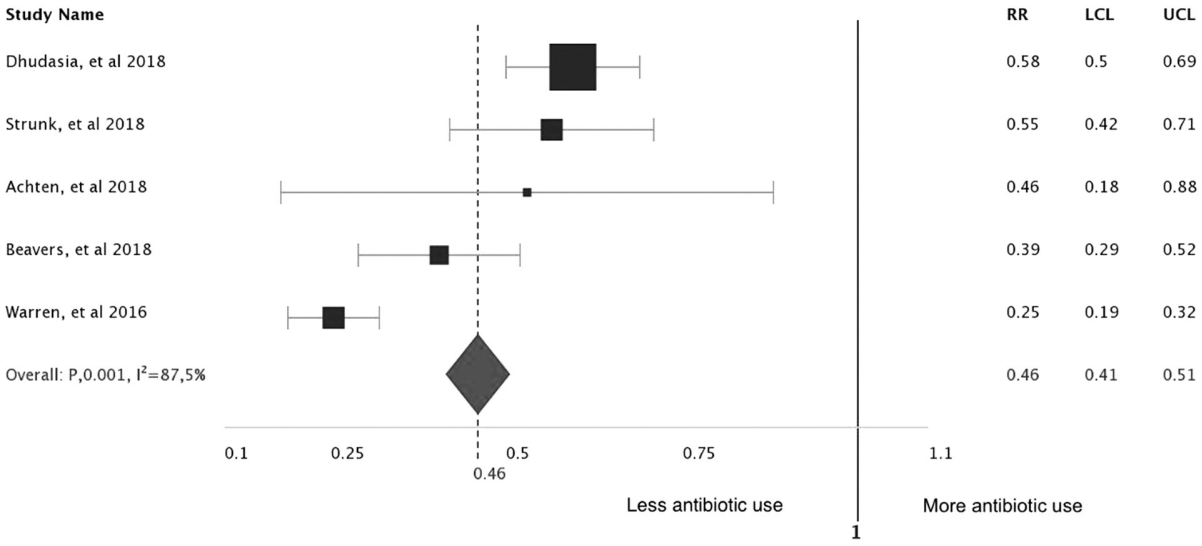

Figure 2. Forest plot of first outcome: use of antibiotics

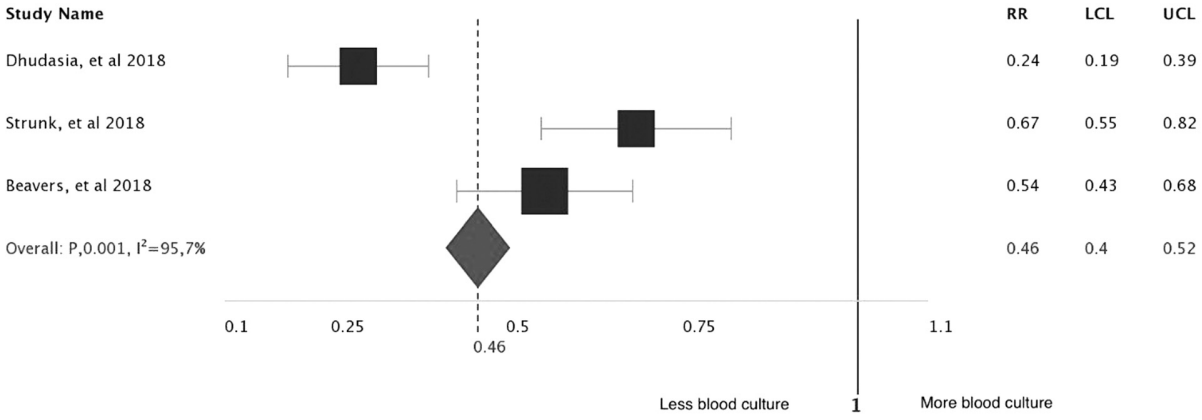

Figure 3. Forest plot of second outcome: blood culture sampling

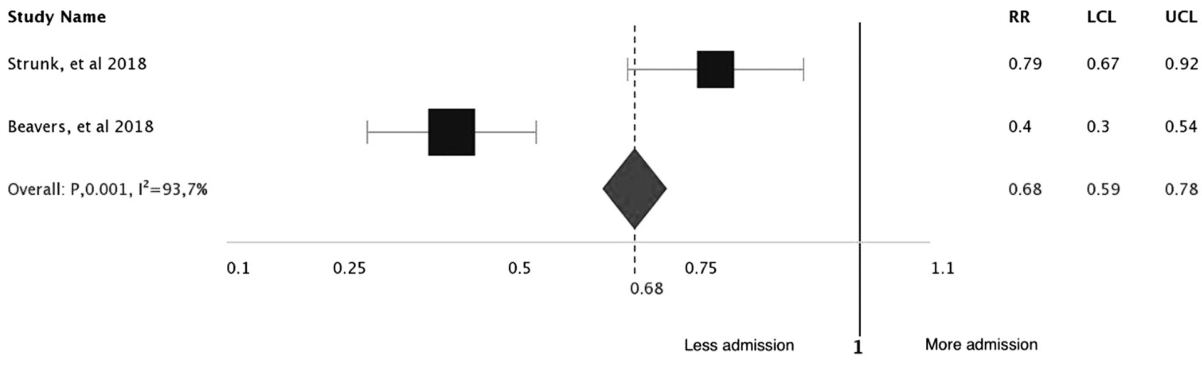

Figure 4. Forest plot of third outcome: higher level neonatal care admission

as the number of studies was inadequate. High heterogeneity level between studies was not an issue since all of the studies had an effect size of less than 1 (including the upper limit of 95% confidence intervals).

Discussion

This study was the first meta-analysis to assess the role of the neonatal sepsis calculator to support antibiotic stewardship since its establishment in 2011 and validity study in 2016. By analyzing results from studies across countries with different EOS incidence and different standards of neonatal care quality, the result of this meta-analysis gave us assurance of the applicability, benefits, and safety of the neonatal sepsis calculator implementation. This meta-analysis would also help both neonatologists and obstetricians in decision-making during daily clinical practice.

Our analysis showed that inappropriate antibiotic administration was decreased after using the neonatal sepsis calculator. The pooled relative risk of "inappropriate antibiotic administration" was less than 0.5. As such, this finding provides marked evidence that the sepsis calculator significantly guided clinicians to implement antibiotic stewardship. Likewise, clinicians became more confident to not administer antibiotics in doubtful clinical situations, notably with an estimated quantification of risk provided by this calculator. It is also possible that clinicians' tendency to give empirical antibiotics would be altered in the near future with greater acceptance of this calculator.[38]

This result was also reiterated by some studies reporting shorter antibiotic use after sepsis calculator implementation (<53 hours).[35] Previously, an international survey showed that 45% of clinicians administer antibiotics if the laboratory result is abnormal, rising to 99% in high-risk situations. Most of them (56%) continued antibiotics to 5-7 days. Since the neonatal sepsis calculator provides guidance, an inappropriate treatment of EOS should be reduced in the near future. Lower inappropriate antibiotic administration could reduce the emergent antimicrobial resistance rate and harmful effect on the neonatal microbiome. In contrast, inappropriate antimicrobial use led to late-onset neonatal sepsis.[39-40] Lower antibiotic administration also minimized intravenous (IV) access and lowered the adverse events from excessive drug administration.[41] Moreover, studies have also reported that inappropriate antibiotic administration during infancy increased the risk of developing asthma,[42] autoimmune disease,[43] and obesity[44] in the future.

We found that the blood culture sampling rate was significantly reduced with the implementation of the sepsis calculator. The amount of blood, taken merely to perform blood cultures, was relatively large for a newborn, especially for preterm infants. Not only did decreased blood sampling allow avoidance of a painful venous puncture, it also reduced the unnecessary cost and hospital stay just to wait for the results. This was imperative since blood culture sampling rate was routinely done, regardless of culture results' low positivity rate and lack of usefulness in altering our approach in patient management. A multicenter survey of neonatal units showed that the most ordered laboratory exam in newborns with suspected EOS was complete blood count (97.2%), followed by blood culture (80.3%), and C-reactive protein (29.6%).[45] Therefore, blood sampling for culture was an important issue and any reduction could improve antibiotic stewardship.

Some literature about biomarkers in EOS also stated that gestational age and other physiologic processes, including maternal and perinatal factors, influenced the levels of CRP in the first three days of life after birth.[46-49] With a cutoff value of 10 mg/L and when combined with other biomarkers, CRP had a superior diagnostic accuracy.[46] The CRP response was noted to be higher in gram-negative than in gram-positive infections.[46] By lowering the blood culture rate, the use of other laboratory parameters such as CRP, procalcitonin (PCT), or immature-to-total neutrophil ratio (I/T ratio) could be optimized, as those were faster and simpler tests that involved less blood volume.[4] These results could then lead to optimization of laboratory examinations directly during the observation phase when an infant presented a clinical deterioration later, after 12 hours of life.

Sepsis was the most common diagnosis that led to newborn neonatal unit admissions, mainly the NICU. The presence of sepsis, even only in suspected cases, indeed warranted admission to a higher level of neonatal care. In our review, the pooled risk ratio

for higher neonatal care was 0.68, following the use of neonatal sepsis calculator. Through a more detailed analysis, this ratio was higher than antibiotic administration (RR 0.46), meaning that although not given antibiotics, several clinicians still decided on a higher neonatal care level to observe high-risk newborns. We found that reduced neonatal care admission had several consequences both for the newborn and parents. Higher neonatal care admission led to longer hospital stay, raised parental anxiety, and created a huge burden in terms of health care cost.[41] Moreover, these factors could lead to disruption in maternal-infant bonding and delayed early breastfeeding.[41] In developing countries, an admission to A neonatal unit (especially NICU) also increased the risk of nosocomial infection, known as late-onset neonatal sepsis.

The goal of all existing approaches in neonatal sepsis risk assessment is newborn safety. The devastating effects of neonatal sepsis on morbidity and mortality prompt clinicians to start antibiotic regimens as soon as there is a suspicion of sepsis. Several journals reported cases of neonatal sepsis who were not given antibiotic recommendation by the sepsis calculator. However, those studies were retrospective chart reviews. We preferred to not rely on chart review studies, since the high bias between clinical and calculator decision. A previous study also evidenced an improvement in antibiotic stewardship through close monitoring of at-risk newborns only by physical examination.[50] We also noted that the neonatal sepsis calculator could not be used as a single parameter to predict EOS without considering laboratory or routine physical examination results. Further multicenter research on the calculator's safety is needed.

Based on the evidence provided above, we concluded that the neonatal sepsis calculator was ready to be implemented in daily clinical practice. An added benefit was that this calculator could be implemented at no extra cost. It led to a robust improvement in antibiotic stewardship in the neonatal unit and did not cause any potential harm to newborns. This neonatal sepsis calculator also guided clinicians to a more efficient decision-making process, especially in doubtful and dilemmatic situations when facing suspected early-onset neonatal sepsis, for example, in "well-appearing" babies born from mothers with suspected CAM.[17] The calculator also decreased improper diagnosis of maternal CAM, since merely elevated maternal temperature sometimes led clinicians to CAM diagnoses. Frequent re-evaluations, mainly of clinical findings, were necessary in newborns who received a no antibiotic recommendation, since EOS could develop anytime during the first 72 hours of life.[44] Kuzniewicz *et al.* reported that 50% of newborns with culture-proven EOS were asymptomatic at birth.[29] Wortham *et al.* found that 22% of full term neonates with culture-proven EOS and CAM exposure remained asymptomatic at 72 hours after birth and 28% presented no signs of sepsis within 6 hours after birth.[51]

The main obstacle reported during the implementation of the sepsis calculator was clinician compliance in using such a real-time, decisive tool. This change of habit takes time and should never be rushed. In addition, it requires training, resources, manpower, and an uptick in provider workload to compensate for any medical error resulting from a miscalculation of EOS risk.

This meta-analysis yielded a favorable result for neonatal sepsis calculator implementation, However, several aspects have not been investigated. Some potential further studies would be about the type of antibiotics used, time to switch to stronger antibiotics, and the duration of antibiotic administration. Indirectly, these components affect both newborn length of stay in the hospital (shorter duration reduced the risk of late-onset neonatal sepsis) and cost during hospitalization. Another potential field for further study is the post-discharge analysis of infants not receiving antibiotics based on the calculator recommendation, although a previous study in *Kaiser Permanente Northern California* (KPNC, an original sample population of neonatal sepsis calculator) reported similar readmission rates between the use of CDC guidelines and the neonatal sepsis calculator.[29] A 'wash-out' period was recommended for any further study to investigate the efficiency of using this calculator to improve data quality when conducting a historical cohort. This approach was also done by KPNC from 2012 to 2014 before they completely implemented this calculator.

Risk stratification using the neonatal sepsis calculator is an effective way to improve antibiotic stewardship in the neonatology unit. By reducing

the administration of inappropriate antibiotics, blood sampling for cultures, and admission to higher-level neonatal care, this calculator can help clinicians to evaluate and make decisions for EOS treatment. A prospective meta-analysis in upcoming years is needed to give stronger evidence from a high quality study on the impact of the neonatal sepsis calculator on antibiotic stewardship.

Conflict of Interest

None declared.

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Supplementary Appendix 1. Excluded studies for meta-analysis and reasons for exclusion

Author (year of publication)	Reason for exclusion
Shakib *et al.* (2015)	Excluded patients admitted to NICU; no exact proportion of newborn recommended to receive antibiotic by neonatal sepsis calculator
Kerste *et al.* (2016)	A chart review; unable to calculate relative risk since all sample receive antibiotics
Carola *et al.* (2017)	A chart review; all samples were given antibiotics, underwent blood culture, and admitted to NICU
Money *et al.* (2017)	A chart review where all samples were given antibiotics and admitted to NICU

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Case Report

Hunter syndrome with hyperthyroidism: a 16-month follow-up case report

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Mucopolysaccharidosis (MPS) is a rare genetic disorder caused by a deficiency in the activity of lysosomal enzymes required for glycosaminoglycan (GAG) degradation. An accumulation of GAG in many organs results in progressive cellular damage, and clinically results in joint stiffness, airway and cardiac as well as, mental and hearing impairments. Incidence of MPS was reportedly 2.04 per 100,000 live births, but varies depending on type and region. In Taiwan, MPS type II was the most prevalent MPS, with an incidence of 1.07 per 100,000 live births.[1] MPS is generally inherited in an autosomal recessive pattern, with the exception of MPS II, which is X-linked recessive.[2] There are seven types of MPS (MPS I, II, III, IV, VI, VII, and IX), based on enzyme deficits.[3] The types of MPS with their enzyme deficiencies are listed in **Table** 1.

Mucopolysaccharidosis shows wide clinical heterogenity, and is, therefore, difficult to diagnose. Skeletal involvement in MPS include coarse face, loss of joint range of motion, restricted mobility, and slowed growth leading to short stature. Other signs and symptoms include vision and hearing loss, recurrent respiratory infections, obstructive sleep apnea, hepatosplenomegaly, umbilical and inguinal hernia, hydrocephalus, spinal cord compression, and cognitive impairment.[2,4] Patients with suspected MPS should have urinary GAG laboratory testing and enzyme activity assays in tissue (blood or fibroblasts). Urinary elevation of GAG, as compared with GAG levels expected in age-matched normal subjects, is the first diagnostic approach. The definitive specific diagnosis for MPS is based on enzyme activity assays from cultured fibroblasts, leukocytes, plasma, or serum.[2,5,6] The MPS patients require multidiciplinary subspeciality management, including ENT, orthopedics, cardiology, pulmonary, growth and development, and physiotherapy. Specific treatments for MPS are hematopoietic stem cell transplantation (HSCT) and enzyme-replacement therapy (ERT) with recombinant human enzymes for MPS I, II, and VI.[3,6,7,8] Life expectancies in MPS may vary among types, but generally are markedly reduced. Patients with MPS III and VII and severe forms of MPS I and MPS II have mental retardation. Patients with MPS II usually survive until only the second decade of life, with respiratory failure as the leading cause of death (56%), followed by cardiac failure (18%).[9,10] **[Paediatr Indones. 2018;58:317-22; doi: http://dx.doi.org/10.14238/pi58.6.2018.317-22]**

Keywords: *Hunter syndrome; mucopolysaccharidoses; hyperthyroidism*

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Table 1. The types of MPS

Type	Enzyme deficiency	GAGdeposits	Gen loci
I (Hurler, Hurler Scheie, Scheie syndrome)	α-L iduronidase (IDUA)	Heparan sulphate, dermatan sulphate	4p 16.3
II (Hunter syndrome)	iduronate-2-sulphatase (I2S)	Heparan sulphate, dermatan sulphate	Xq28
III (Sanfilippo syndrome)	A: heparan N-sulphatase B: N-acetyl- α-glucosaminidase C: acetyl-CoA α-glucosaminidase D: N-acetylglucosamine 6-sulphatase	Heparan sulphate	A: 17q25.3 B: 17q21 C: 8p11.1 D: 12q14
IV (Morquio syndrome)	A: N- acetylgalactosamine 6- sulphatate sulphatase B: β-galactosidase	A: Keratan sulphate, chondroitin sulphate B: Keratan sulphate	A: 16q24.3 B: 3p21.33
VI (Maroteaux-Lamy syndrome)	N-acetylgalactosamine 4-sulphatase	Dermatan sulphate, chondroitin sulphate	5q11-q13
VII (Sly syndrome)	β-glucoronidase	Dermatan sulphate, chondroitin sulphate, heparan sulphate	7q21.11
IX (Natowicz syndrome)	Hyalurinidase	Hyaluronan	3p21.3-p2 12

The Case

A boy aged 7 years and 10 months was a referral case to Sardjito Hospital, Yogyakarta, who was diagnosed with hyperthyroidism and global developmental delay in April 2013, due to tachycardia, chronic diarrhea, regressed motor ability, and cognitive impairment. Thyroid function test showed FT4 elevation (70 pmol) and low TSH (0.064 μIU/mL). After 1 year of propylthiouracil (PTU) treatment, the patient became euthyroid (FT4: 0.89 pmol and TSH 0.79 μIU/mL) so medication was stopped. The patient returned to Sardjito Hospital on May 2014 with restlessness, inability to sleep for two days, and diarrhea (three times/day). Physical examination showed manifestations of mucopolysaccharidosis, i.e., coarse face, short stature, hepatosplenomegaly, joint stiffness, and cognitive imparment. His coarse face is shown in **Figure 1**. The patient was diagnosed with hyperthyroidism (relapsed), intellectual disability, and suspected MPS. Laboratory results for thyroid function were FT4 1.56 μIU/mL and TSH 0.207 μIU/mL. Radiological examinations were done to evaluate for adenoid hypertrophy, joint stiffness, cardiomegaly, and vertebral deformities. This patient had adenoid hypertrophy (**Figure 1**), joint stiffness (**Figure 2**), and claw hands (**Figure 3**, **Figure 4**). Echocardiography revealed mild-moderate aortic insufficiency and mitral insufficiency. The patient also had profound bilateral distal neural hearing loss. Urinary GAG and enzyme activity assays were performed at the University of Taiwan Laboratory. Urinary GAG analysis showed

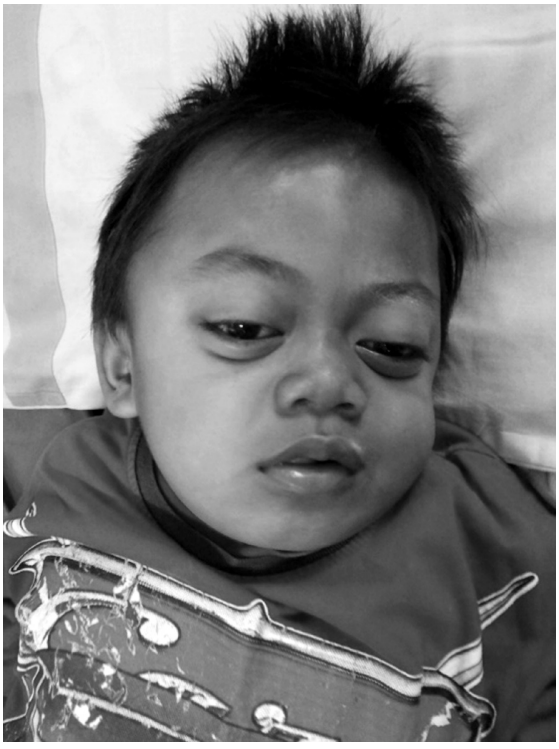

Figure 1. Patient with coarse facial features

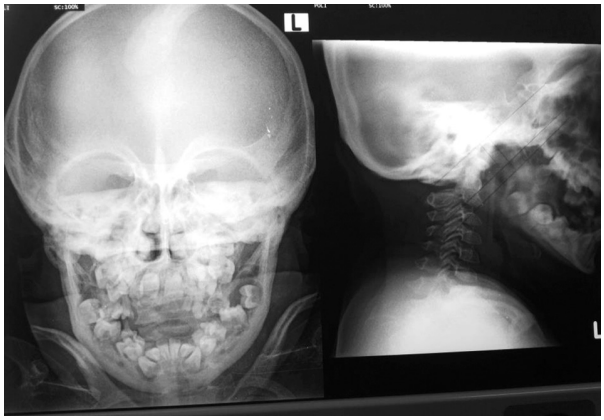

Figure 2. X-rays revealing adenoid hypertrophy

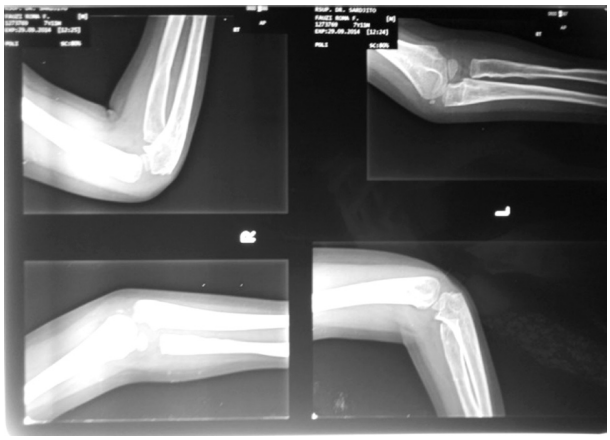

Figure 3. X-rays of the elbow joint

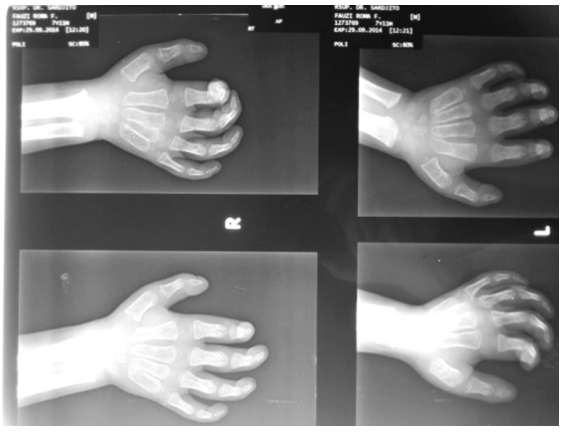

Figure 4. X-rays indicating claw hand

elevated GAG of 685.57 mg GAG/g creatinine, in comparison with the age-matched value of 35.74 (10.77-77.50) mg of GAG/g creatinine. There was low plasma α-iduronate sulphatase activity, but normal activity in his leukocytes. The enzyme activity results are listed in **Table 2** and **Table 3**. The final diagnosis for this patient was MPS type II (Hunter syndrome), hyperthyroidism, mild-moderate aortic insufficiency, mitral insufficiency, intellectual disability, and profound bilateral distal neural hearing loss. The patient received palliative therapy for MPS. He also received propylthiouracyl for hyperthyroidism, ACE-I for cardiac insufficiency, and physiotherapy. Psychosocial testing performed by a psychologist revealed that this 7-year-old patient had a mental age below 2 years, communication skill of a 3-month-old,

Table 2. Enzyme activity assays in the patient's plasma

Enzymes	Results	Normal values
4B: β-galactosidase	9.31	10.22 ± 5.03 nmol/mg prot/30min
2: α-iduronate sulfatase	0.76	496.3 ± 165.7 nmol/mg prot/4 hrs
3B : α-hexoaminidase	721.08	320.4 ± 131.3 nmol/mg prot/17 hrs

Table 3. Enzyme activity assays in the patient's leuk ocytes

Enzymes	Results	Normal values
1-α-iduronidase	26.24	41.8 ± 15.9 nmol/mg prot/hrs
2-α-iduronate sulfatase		28.8 ± 11.3 nmol/mg prot/4 hrs
3A-heparan sulphamidase		4.6 ± 2.2 nmol/mg prot/24 hrs
3B-α-hexoaminidase		17.2 ± 6.4 nmol/mg prot/17 hrs
3C-acetyl-CoA: a-glucosaminide N acetytransferase	2.32	7.5 ± 2.2 nmol/mg prot/17 hrs
3D-N-acetylglucosamine-6-sulphate sulphatase		7.76 ± 5.15 nmol/mg prot/24 hrs
4A-galactose-6-sulphate sulphatase	177.72	158.9 ± 82.8 nmol/mg prot/17 hrs
4B-β-galactosidase	93.57	93.85 ± 28.75 nmol/mg prot/30min
4MLD: arylsulfatase A	76.53	76.6 ± 29.9 nmol/mg prot/hrs
6: arylsulfatase B	85.76	92.3 ± 49.6 nmol/mg prot/hrs
7: β-glucuronidase	72.58	110.3 ± 32.4 nmol/mg prot/hrs

daily activity skill of a 10-month-old, social skill of a 2-month-old, and motor skill of an 8-month-old.

We followed up this patient for 16 months to see the natural history of MPS with hyperthyroidism and its complications, including growth, physical performance, cardiac involvement, and sleep disturbance. This patient demonstrated a flat pattern of growth, with no significant increase in weight and height. On the initial examination, the patient's weight was 20.0 kg and height was 103.0 cm. Upon monthly follow-up visits, his body weight was stable at 20.0 kg, i.e., between -3 SD and -2 SD weight-for-age, according to the 2006 WHO Child Growth Standards.[11] At the end of follow-up, the patient's height was 105.0 cm, an increase of 2 cm. However, it was still below -3SD, according to the *2006 WHO Child Growth Standards* for 8-year-old boys. His BMI was normal.

Cardiac involvement included worsening valvular insufficiency, with aortic insufficiency developing from mild-moderate to moderate, and mitral insufficiency progressing from mild to moderate, although the patient routinely consumed an angiotensin converting enzyme inhibitor (ACEI) for 4 months. There were no signs or symptoms of heart failure in this patient. However, towards the end of his life, he received diuretics for suspected heart failure. The first respiratory problem was obstructive sleep apnea syndrome (OSAS) caused by adenoid hypertrophy, which resulted in sleep complications. However, his family refused an adenotonsillectomy due to worries of anaesthetic complications. This patient did not suffer from pneumonia until his last follow-up visit. After having a cough for 5 days, the patient was hospitalized for pneumonia. His condition worsened with seizures without fever that occurred on the 1st day of hospitalization. He required intubation. The patient was never extubated because of excessive saliva production. The patient also suffered from heart failure, and unfortunately he died at the age of 9 years, with respiratory failure as the cause of death after 30 days of hospitalization.

The patient's quality of life was measured with *PedsQL version 4.0* for parental perception. His scores were 25 for initial and final monitoring, indicating that he had physical, social, and emotional problems. The lack of difference between the initial and final score indicated that his quality of life neither improved nor worsened. *The Pediatric Symptoms Checklist* was also performed, with a score above 28 (score=38), i.e., appropriate for psychological impairment.

Discussion

The patient was a boy aged 7 years and 10 months at the beginning of our observation period, and was treated for hyperthyroidism. Suspected MPS was based on clinical presentation and simple investigation. The MPS is a genetic disorder that causes lysosomal enzyme deficiency, leading to a buildup of GAG metabolites in organs. A diagnosis of type II MPS can be based on increased urinary GAG levels and decreased α-iduronate sulfate enzyme activity. A patient with severe intellectual disability is reflective of a severe form of MPS type II.[2,5]

The hyperthyroidism in our patient had improved to euthyroidism following initial treatment. A number of conditions that cause hyperthyroidism in children are congenital hyperthyroidism, Graves' disease, toxic nodular thyrotoxicosis, toxic adenoma, thyroiditis, follicular carcinoma, and TSH-producing tumor of hypophysis. However, in this patient, the etiologic tracking of hyperthyroidism was not done, as the main suspected cause was MPS type II. In MPS, metabolites tend to accumulate in the thyroid gland and brain leading to endocrine disorders.[12,13] To our knowledge, there have been no reports that hyperthyroidism can occur in MPS. However, hypothyroidism is generally caused by panhypopituitarism.[12]

The joint deformity and stiffness in the patient was routinely treated by physiotherapists. Limitations and abnormalities of the bones and joints appearing in MPS require special attention, as they may result in accidents that can aggravate the condition.[7,13,14] Joint deformity correction in our patient was not undertaken because of his baseline lack of ambulation. Moreover, the risk of anesthesia was perceived to be too burdensome by the family.

Child growth is a good indicator for assessing overall child health, as it may be affected by poor nutritional intake, thyroid hormone insufficiency and growth, abnormal bone metabolism, and chronic disease.[15] According to the *2006 WHO Child Growth Standard*, our patient was severely stunted, underweight with normal weight-for-height. Similarly, a report from Taiwan noted

that the natural growth course of MPS patients is the presence of short stature, macrocephaly, weight above normal, and normal or excessive BMI.[15,16] Children with MPS appear normal at birth, but grow poorly compared to normal children. Around 90% of children presenting with MPS have height-for-age -2SD. In general, at the age of 5-10 years, the child's weight is between ± 2SD and after the age of 10, it is under 2SD.16 Macrocephaly is found at all ages. The MPS patients in Japan aged 18 years or over have BMI above 25, indicating a tendency to obesity. The mechanism of poor growth in MPS is not widely known, but is suspected to be associated with a growth plate disturbance in the form of a decrease in the deposit matrix with decreased osteoblast function, hypertrophic chondrocytes, disorganization of growth disc structure, and GAG accumulation on growth discs. In addition to the above, GAG accumulation is also found in pituitary, thyroid, and testicular glands in children with MPS type II.[16]

Complications that appear in MPS patients include thickening of the heart valve and pneumonia. The common respiratory disorders in MPS are OSAS and recurrent respiratory infections due to airway deformity.[17] Seizures in these patients are suspected to have been induced by MPS, sepsis, or hypoxia that may occur in patients with OSAS. Without enzyme therapy, the life expectancy of patients with MPS type II reaches the age of 7-10 years. Our patient died at 9 years and 2 months of age. He had a 1-year-old sister. Although MPS type II is inherited in an X-linked fashion, the sister still had the possibility of having MPS.[5,9,19] However, we did not perform chromosome analysis of the sister due to parental refusal.

Our patient died of pneumonia. Severe type II MPS patients without enzyme replacement therapy die at the age of 13.2 ± 3.2 years, with respiratory failure (56%) and cardiac failure (18%) as the leading causes of death.[20] Such a condition is exacerbated by heart valve abnormalities. From the parents' information, the patient had delayed control for echocardiography, thus cardiac medication was not given. The condition of pregnant mothers is a barrier to bring the patient to our hospital.

Conflict of Interest

None declared.

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