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Original Article

Retinol binding protein 4, obesity, and insulin resistance in adolescents

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Abstract

Background Obesity is a global problem. Even in poor and developing countries, obesity has reached alarming levels. In childhood, obesity may lead to insulin resistance. Retinol binding protein (RBP4), secreted primarily by liver and adipose tissues, was recently proposed as a link between obesity and insulin resistance. The role of RBP4 in pediatric obesity and its relationship with insulin resistance have not been well elucidated.
Objective To compare RBP4 levels in obese and lean adolescents and to assess for a relationship between RBP4 levels and insulin resistance.

Method This cross-sectional study was conducted in three senior high schools in Padang, West Sumatera, Indonesia. Subjects were adolescents aged 14-18 years, who were obese or normal weight (n=56). We measured subjects' body mass index (BMI) and serum RBP4 concentrations. Insulin resistance was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR) index.

Results Similar RBP4 levels were found in the obese and normoweight groups (P>0.05). Higher RBP4 levels were found in the insulin resistant compared to the non-insulin resistant group, but the difference was not significant (P > 0.05).

Conclusion There is no significant difference in mean RBP4 levels in obese adolescents compared to normoweight adolescents. Nor are mean RBP4 levels significantly different between obese adolescents with and without insulin resistance. **[Paediatr Indones. 2017:57:1-7. doi: 10.14238/pi57.1.2017.1-7]**.

Keywords: *obesity; retinol binding protein 4 (RBP4); insulin resistance*

Obesity in children is one of the most serious challenges of the 21st century. Childhood obesity has reached alarming levels, with prevalences increased from 4.2% in 1990 to 6.7% in 2010.[1,2] In Indonesia, the prevalence of obesity, according to the National Socioeconomic Survey (SUSENAS), has increased in both urban and rural areas.[3] Children who are overweight or obese have an 80% risk of becoming obese as adults.[4,5] Obesity in children can lead to insulin resistance,

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which is a key component of metabolic syndrome.[6] Insulin resistance is responsible for 46.8% of coronary heart disease in type 2 diabetes, 6.2% of non-type 2 diabetes, and 12.5% of the total population in the United States.[3]

Adipose tissue is metabolically active organ secreting a number of signal peptides with various biological functions. These adipokines play an important role in the regulation of adipocyte differentiation, metabolism, and local inflammatory response. Adipokines are also involved in the regulation of systemic fat and glucose metabolism in the brain, liver, and muscle. Secreted adipokines include leptin, tumor necrosis factor-alpha(TNF-alpha), interleukin-6 (IL-6), and chemokine (CC-motif) ligand 2 (CCL2), adiponectin, resistin, omentin, vaspin, visfatin, chemerin and retinol binding protein 4 (RBP4).[4,5] The RBP4 is a lipocalin protein produced in the liver and mature adipose tissues that carry retinol in the circulation.[7,8] Serum RBP4 levels were found to be increased and positively correlated with BMI in obese non-diabetics and diabetics. The RBP4 levels were elevated in subjects with impaired glucose tolerance and diabetes mellitus type 2, but inversely related to insulin sensitivity. It is not clear if a retinol-dependent mechanism is associated with obesity or insulin resistance.[6] The aim of this study was to compare serum RBP4 levels in obese to lean adolescents and to assess for a relationship between RBP4 levels and insulin resistance.

Methods

The subjects for this study were 14 to 18-year-old adolescents (n=56) from three senior high schools in Padang. The children were recruited through direct observation and anthropometric data. Our intent was to enroll 28 normoweight and 28 obese children for the study. Written informed consent was obtained from parents and oral informed assent from the children. The study was approved by the Ethics Committee of the Andalas University Medical School. We took anthropometric data by measuring body weight and heights. Height was measured to the nearest 0.5 cm and weight to the nearest 100 g using a digital balance. After measuring BMI, resting blood pressure was measured by auscultation. Subjects filled questionnaires on family history, diet, and behavior. Subjects provided 8-mL blood specimens by venipuncture in the morning after a 12-h overnight fast. The HOMA-IR index was used to assess subjects for insulin resistance. The present HOMA cut off point for diagnosis of insulin resistance is 3.16. The HOMA cut off point of >2.5 is valid for adults but not for adolescents.[9]

Body mass index (BMI) of the children was calculated as weight (kilograms)/height (meters-squared). Age and gender-specific criteria from the *Centers for Disease Control* (CDC) 2000 were used to classify children as normal weight, or obese (above the 95th percentile). Statistical analysis was performed using *SPSS 17.0 for Windows* software. Non-normally distributed variables were expressed as median (range) and normally distributed data by mean (SD). We used descriptive analysis, T-test, and regression correlation for data analysis. Descriptive analysis was used to describe the mean (SD) for ratio scale data, such as age, body weight, height, BMI, insulin levels, fasting blood glucose levels, HOMA-IR, and RBP4 levels. Analysis T-test was used to assess for differences in the obese and normoweight groups. Results with P values <0.05 were considered to be statistically significant.

Results

Anthropometric and metabolic data of the subjects, by weight classification, are shown in **Table 1**. The mean BMIs were 32.36 (4.00) kg/m2 in the obese group and 21.85 (2.49) kg/m2 in the normoweight group. Median systolic/diastolic blood pressures were significantly higher in the obese group than in the normoweight group (P<0.05).

Mean insulin level was significantly higher in the obese group than in the normoweight group (P<0.05). Also, the mean HOMA-IR value was significantly higher in the obese group than in the normoweight group (P <0.05). The mean RBP4 level was 24.27 (5.32) pg/mL in the obese group and 24.68 (8.10) mg/mL in the normoweight group (P> 0.05).

Obese subjects' anthropometric, metabolic, and insulin resistance data are shown in **Table 2**. Insulin resistance was found in 10/28 adolescents in the obese group, and in none of the normoweight subjects. Median HOMA-IR in the insulin-resistant group was

significantly higher than in the non-insulin resistant group (P<0.05). However, mean RBP4 levels were not significantly different in the insulin resistant and non-insulin resistant groups [26.42 (5.02) pg/mL *vs.* 23.08 (5.23) mg/mL, respectively]. There was also no statistically significant difference in median RBP4 levels between the two groups (P>0.05).

and adolescents. Weight loss was associated with a decrease in insulin concentration and improved insulin sensitivity in adolescents. In a study of 122 adolescents, obese adolescents had more insulin resistance and abnormal lipid profiles compared to lean adolescents.[10] A population-based study conducted in the United States in 4,902 children aged 12-19 years

Table 1. Anthropometric and metabolic data of subjects by weight classification

Characteristics	Obese group (n=28)	Normoweight group (n=28)	P value
Gender, n(%)			
Male	4 (14.28)	13 (46.42)	0.020*
Female	24 (85.72)	15 (53.58)	
Median age (range), years	16 (15-18)	16 (15-18)	0.392**
Family history of obesity, n(%)			
Yes	15 (53.57)	8 (28.57)	0.103**
No	13 (46.43)	20 (71.43)	
Median systolic BP (range), mmHg	120 (100-150)	110 (90-120)	0.004**
Median diastolic BP (range), mmHg	80 (60-100)	70 (60-80)	0.002*
Median random blood glucose (range), mg/dL	78.00 (69-99)	77.5 (68-95)	0.465**
Mean insulin (SD), µIU/mL	12.73 (7.13)	5.65 (3.59)	0.001*
Mean HOMA-IR (SD)	2.63 (1.58)	1.06 (0.61)	0.001*
Mean RBP4 (SD), µg/mL	24.27 (5.32)	24.68 (8.10)	0.827*

*T-test; **Mann-Whitney test

Table 2. Anthropometric and metabolic data of obese subjects by insulin resistance classification

Characteristics	Insulin resistant group (n=10)	Non-insulin resistant group (n-10)	P value
Gender, n			
Male	2	2	0.452*
Female	8	16	
Median age (range), years	16.5 (15-180	16 (15-18)	0.710**
Family history of obesity, n(%)			
Yes	7	8	0.184*
No	3	10	
Median BMI (SD), kg/m^2	34.26 (4.49)	31.10 (3.39)	0.061*
Mean systolic BP (SD), mmHg	127 (14.18)	119.44 (15.13)	0.207*
Mean diastolic BP (SD), mmHg	85 (10.80)	79.44 (11.61)	0.225"
Median random blood glucose (range), mg/dL	85 (71-94)	77 (69-99)	0.064*
Mean insulin (SD), µIU/mL	20.38 (5.61)	8.45 (3.26)	0.001*
Mean RBP4 (SD), µg/mL	26.42 (5.02)	23.08 (5.23)	0.114*

*T-test; **Mann-Whitney test

Discussion

Insulin resistance, as described by the HOMA-IR index, was significantly increased in the obese group compared to the normoweight group. The incidence of insulin resistance in obese adolescent subjects was 35.71%. The relationship between adiposity and insulin resistance has been reported in adults

reported that the incidence of insulin resistance in obese adolescents was 52.1%.[11] Similarly, Gatenbein et al. reported a significant increase in HOMA-IR in obese adolescents compared to controls.[12]

Insulin resistance causes hyperinsulinemia which can lead to glucose intolerance, atherogenic dyslipidemia, hypertriglyceridemia, and increased blood pressure. Systolic and diastolic blood pressures were significantly higher in the obese group compared

to the normoweight group (P<0.05). Graham *et al.* also reported an increase in systolic blood pressure in obese adolescents with increased HOMA-IR values, compared to the control group. Insulin acts to increase renal sodium retention and free water clearance. Insulin resistance also increases the activity of the sympathetic nervous system and stimulates the growth of vascular smooth muscle. Insulin levels were found to be significantly higher in patients with essential hypertension compared with normotensive controls.[13]

We found no statistically significant differences in the RBP4 levels in obese and normoweight adolescents. In the obese group, RBP4 levels were higher in adolescents with insulin resistance compared to obese adolescents without insulin resistance, although this difference was not statistically significant (P>0.05). Several factors play a role in the pathogenesis of obesity-related insulin resistance including increased levels of free fatty acids, hormones, and cytokines released by the adipose network.[12] The relationship between RBP4 and insulin resistance in cross-sectional studies is still unclear. However, several studies in adults showed a significant association between RBP4, obesity and metabolic syndrome.[14]

A study conducted on 42 adolescents aged 14-18 years in Canada found that RBP4 levels were higher in obese children than in thin children. Furthermore, higher RBP4 levels were associated with insulin resistance, but also by the inflammation factor, C-reactive protein (CRP).[15] Reinehr *et al*. conducted a longitudinal study over one year by examining RBP4 levels in obese children before and after weight loss due to exercise intervention, behavior, and nutrition. They found that RBP4 levels were higher in the obese group, but decreased after weight loss. In addition, RBP4 levels at puberty were higher than at the age of puberty.[13,15]

A previous study examined RBP4 and its relationship to insulin resistance in 49 American children at puberty. This study showed that there were no significant differences in mean RBP4 levels in the age of puberty between the sample and the control groups. RBP4 was correlated with levels of triglycerides, but not related to IMT.[16]

Graham *et al.* conducted a study to measure RBP4 levels, insulin resistance, and metabolic components in three groups: lean, obese, and diabetic. They found that RBP4 levels correlated with obesity, impaired glucose tolerance, and diabetes mellitus type 2, however, RBP4 levels were also elevated in non-obese and non-diabetic subjects who had a family history of obesity and type 2 diabetes. There were 15 non-obese adolescents in the group who had higher RBP4 levels above the mean value of 24.68 (8.10) ug/dl, eight (53.33%) of whom had a family history of obesity.[13]

Several other studies also reported that RBP4 levels were not significantly different in obese and non-obese children.[15-17] A previous study conducted on 80 obese girls aged 9-15 years who were divided into a control group, nutrition, obesity and severe obesity year in Greece. They examined levels of RBP4 and lipocalin-2 and their relationships to high-sensitivity C-reactive protein (hs-CRP), leptin, and adiponectin. Mean RBP4 level was 24.8 (1.3) mg/dL in the control group and 24.9 mg/dL in the obese group.16 Similarly, we found that mean RBP4 levels were 24.68 (8.10) mg/dL in the non-obese group and 24.27 (5.32) mg/dL in the obese group. In addition, Kanaka-Gatenbein *et al.* reported that levels of RBP4 and lipocalin-2 inversely correlated with BMI and hs-CRP, while leptin positively correlated with IMT.15 Janke et al. conducted a study of 74 obese, overweight, and underweight subjects and found that RBP4 mRNA was downregulated in the subcutaneous adipose network, and circulating RBP4 levels did not differ among the three groups.[17]

The correlation of serum RBP4 levels and plasma insulin levels indicates that the expression of RBP4 in adipose tissue may be a direct consequence of hyperinsulinemia. However, subjects with type 2 diabetes had lower fasting insulin levels than subjects with impaired glucose tolerance with the same degree of insulin resistance, but with the same levels of RBP4. In addition, RBP4 and insulin levels were not related in subjects who did not experience an increase in insulin sensitivity after exercise. Therefore, the primary reduction of plasma insulin levels alone does not determine serum RBP4 levels. However, there may be a threshold at which plasma insulin is permissive for increased expression of RBP4 in adipocytes, because RBP4 levels decreased in subjects with newly onset type 1 diabetes and returned to normal after insulin treatment.[13]

In several pediatric studies, the major biological

Obese subjects' anthropometric, metabolic, and insulin resistance data are shown in **Table 2.** Insulin resistance was found in 10/28 adolescents in the obese group, and in none of the normoweight subjects. Median HOMA-IR in the insulin-resistant group was significantly higher than in the non-insulin resistant group ($P<0.05$). However, mean RBP4 levels were not significantly different in the insulin resistant and non-insulin resistant groups [26.42 (5.02) pg/mL *vs.* 23.08 (5.23) mg/mL, respectively]. There was also no statistically significant difference in median RBP4 levels between the two groups ($P>0.05$).

Discussion

Insulin resistance, as described by the HOMA-IR index, was significantly increased in the obese group compared to the normoweight group. The incidence of insulin resistance in obese adolescent subjects was 35.71%. The relationship between adiposity and insulin resistance has been reported in adults and adolescents. Weight loss was associated with a decrease in insulin concentration and improved insulin sensitivity in adolescents. In a study of 122 adolescents, obese adolescents had more insulin resistance and abnormal lipid profiles compared to lean adolescents.[10] A population-based study conducted in the United States in 4,902 children aged 12-19 years reported that the incidence of insulin resistance in obese adolescents was 52.1%.[11] Similarly, Gatenbein et al. reported a significant increase in HOMA-IR in obese adolescents compared to controls.[12]

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determinant of serum RBP4, namely vitamin A status, was not measured.[14] The RBP4 is specific to the transport protein for blood retinol. Retinol intake disorders and vitamin A status affect the release of RBP4 by the liver and circulating RBP4 in the blood. It remains unclear whether the relationship between RBP4 and insulin sensitivity depends on retinol.[14] Aeberli et al. examined the levels of serum RBP4 and retinol, as well as the ratio of RBP4 to retinol and vitamin A intake in 79 Swiss children. Subject groups were normoweight, overweight, and obese. They examined the relationship of these variables to insulin resistance, sub-clinical inflammation, and metabolic syndrome. They found that only 3% of children had low vitamin A status. RBP4 and RBP4/SR significantly correlated with serum triglycerides and RBP4/SR correlated with fasting insulin. The ratio of RBP4/SR was more strongly correlated with obesity, central obesity, and metabolic syndrome components compared to serum RBP4.[14]

Many confounding factors accompany studies of this kind, ranging from methodology to sampling. Retinol status, iron status, and renal function also affect RBP4 levels, but not many studies have linked these factors. The ratio of retinol to RBP4 is influenced by the state of deficiency and obesity. But in the case of obesity, synthesized RBP4 released by adipose tissue into circulation does not bind to retinol.[18] Vitamin A deficiency can interfere with iron metabolism and aggravate anemia. In recent years, increased iron intake and increased iron reserves have been recognized to be significant contributors to insulin resistance in the general population and in patients with type 2 diabetes.[19] In contrast, iron supplementation significantly increases plasma retinol and RBP4.19 Fernandez-Real *et al.* conducted a study on 132 non-diabetic, middle-aged men, by checking their iron status and RBP4. They found that RBP4 levels positively correlated with serum ferritin and RBP4 levels decreased after iron depletion. They concluded that RBP4 plays an important role in relation to the resistance RBP4 insulin.[19] However, studies in children and adolescents have been limited. The relationship of RBP4 with some metabolic parameters has been studied in detail, but little is known about the relationship of this adipokine with kidney function, especially in patients with decreased (mild-moderate) glomerular filtration rate (GFR). Ziegelmeier *et al.* determined serum RBP4 levels in 58 adult patients on chronic hemodialysis (32 diabetic and 26 non-diabetic) and 59 control subjects (29 diabetic and 30 non-diabetic). A GFR of 50 mL/min and RBP4 correlated with clinical and biochemical kidney function, glucose and lipid metabolism, as well as inflammation in both groups. They found that RBP4 was negatively correlated with GFR, and RBP4 levels were higher in patients with decreased kidney function.[10] In our study, we did not check the statuses of retinol, iron, and kidney function due to cost and the limited number of blood specimens taken.

The adipokine, RBP4, is promising in humans as a link between adiposity, insulin resistance, type 2 diabetes mellitus, and metabolic syndrome components. However, many studies on the relationship and/or causality due to RBP4 expression in the above circumstances have not been fully explained.[8]

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Original Article

Transcutaneous bilirubinometry to estimate total serum bilirubin in neonatal jaundice

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Abstract

Background The gold standard for diagnosis of neonatal jaundice is total serum bilirubin (TSB) measurement. This method, however, is invasive, painful, and costly in terms of workload, time, and money. Moreover, repeated blood sampling may lead to significant blood loss, which is of particular concern in preterm infants. To overcome these drawbacks, non-invasive methods of bilirubin measurement have been proposed. Transcutaneous bilirubinometry (TcB) determines the yellowness of the subcutaneous tissue of a newborn infant by measuring the difference between optical densities for light in the blue and green wavelength regions.

Objective To evaluate the accuracy of transcutaneous bilirubinometry for estimating TSB levels in neonatal jaundice.

Methods Subjects were infants aged < 28 days with jaundice who had never been treated with phototherapy or exchange transfusion. The study was done from February to July 2016 in Mohammad Hoesin Hospital. Subjects underwent transcutaneous bilirubin (TcB) and TSB assays, with a maximum interval of 15 minutes between tests.

Results One hundred fifty patients were included in this study. The TcB values > 5 mg/dL were correlated to TSB > 5 mg/dL, with 100% sensitivity and 83.3% specificity. This cut-off point was obtained from a receiver-operator characteristic (ROC) curve with AUC 99.3% (95%CI 97.9 to 100%; P< 0.001).The correlation coefficients (r) for TSB and TcB measurements on the forehead were 0.897 (P<0.001).

Conclusion Transcutaneous bilirubinometry can be used to accurately estimate TSB levels in neonatal jaundice, and may be useful in clinical practice as a non-invasive method to reduce blood sampling. **[Paediatr Indones. 2017:57:8-11. doi: 10.14238/pi57.1.2017.8-11]**.

Keywords: *JM-105; transcutaneous bilirubin; total serum bilirubin*

Neonatal jaundice is often found in term and preterm infants. Most cases donot require treatment, but because of the potential toxicity of bilirubin, all newborns should be monitored.[1-4] In areas with limited health facilities, serum bilirubin concentration may be assessed using Kramer's scale, but the gold standard remains to be TSB measurement. This method, however, is invasive, painful, and costly, in terms of workload, time, and money. Moreover, repeated blood sampling may lead to significant blood loss, which is of particular concern in preterm infants. To overcome these drawbacks, non-invasive methods of bilirubin measurements have been proposed. Transcutaneous bilirubinometry has been shown to be correlated to serum bilirubin concentration in infants.[5-9]

The new Drager Jaundice Meter JM-105 uses 2 wavelengths and a dual optical path system. The operational principles have been described in detail by Yasuda *et al.* who used the JM-103 model, the predecessor to the JM-105.10 The jaundice meter has two optical beams, one of which reaches only

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the shallow areas of subcutaneous tissue, while the other penetrates the deeper layers. The differences between the optical densities are detected by blue and green photocells. Bilirubin accumulated primarily in the deeper subcutaneous tissue should decrease the influence of other pigments in the skin, such as melanin and hemoglobin. In a study of 77 Japanese infants, TcB levels with the JM-103 had a strong correlation well toTSB levels and performed better than the JM-102 model.[10]

There are significant variations among the different instruments for TcB measurement. When TcB is used as a clinical substitute for TSB, the new instruments should always be compared to TSB performed by the laboratory, in order to ensure good correlation.11 We aimed to evaluate the accuracy of transcutaneous bilirubinometry at Mohammad Hoesin Hospital, Palembang for the following reasons; (1) for early estimation of TSB so that invasive blood sampling procedures can be reduced; and (2) because to date, such a study has not been done in Mohammad Hoesin Hospital, Palembang, South Sumatera.

Methods

This study was conducted in the maternity ward, intermediate care, and the neonatal intensive care unit (NICU) at Mohammad Hoesin Hospital, Palembang, from February to July 2016. Subjects were infants aged < 28 days with jaundice which was measured by Kramer's scale. We excluded patients who had been treated with phototherapy or exchange transfusion, or whose parents did not consent to participation. The study was approved by the Medical Ethics Committee of Mohammad Hoesin Hospital. Subjects' parents provided informed consent.

The TcB and TSB assays were performed for all patients, with a maximum interval of 15 minutes between tests. All TcB measurements were performed by one investigator, using transcutaneous bilirubinometry (Drager Jaundice Meter, Minolta JM-105). The measurements were obtained from the forehead of the infants, while they were lying in a supine position. The fiber optic probe was placed against the forehead and gentle pressure was applied to exert even contact between the probe and the skin. The TSB assay was performed using a diazo-based method12 in the clinical chemistry laboratory of Mohammad Hoesin Hospital, Palembang, which was calibrated daily before use, in accordance with the manufacturer's instructions. Demographic data, TcB, and TSB values were analyzed using SPSS version 17.0. Pearson's linear regression analysis was used to find a correlation coefficient between TcB and TSB. We assessed cut-off point, sensitivity, and specificity using ROC curve analysis.

Results

One hundred and fifty neonatres were included in this study. The male to female ratio was 1: 1.2. **Table 1** shows the general characteristics of subjects. The majority of subjects were in < 7 day-old age group (139 neonates, 92.7%). The majority of subjects had fullterm gestational age (105 neonates, 70%) and normal birth weight of 2,500-4,000 grams (94 neonates, 62.7%). Most subjects had TSB > 5 mg/dL (144 neonates, 96%) and TcB > 5 mg/dL (145 neonates, 96.7%).

Table 2 shows the subjects' TSB and TcB concentrations. The TSB levels ranged from 4.15 to 21.66 mg/dL [mean 12.32 (SD3.4) mg/dL] and TcB levels ranged from 4.03 to 19.50 mg/dL [mean 13.05 (SD 3.5) mg/dL].

The ROC curve had an area under the curve (AUC) of 99.3% (95%CI 97.9 to 100%; P<0.001). A TcB cut-off point of > 5 mg/dL had 100% sensitivity, 83.3% specificity, 99.3% positive predictive value, and 100% negative predictive value.

Figure 1 shows the relationship between TSB and TcB, as represented by the linear equation y=0.9+0.87*x. This equation was able to correctly predict TSB with an accuracy of 80% (r2=0.805). The correlations between TSB and TcB were found to be significant and close to Pearson's correlation coefficient (r) for TSB and TcB measurements (r=0.897; P<0.001).

Discussion

A number of studies have demonstrated the possibility of predicting serum bilirubin concentration in neonates by the spectral reflectance from the

Table 1. Subjects' characteristics

Characteristics	N=150
Gender, n(%)	
Male	68 (45.3)
Female	82 (54.7)
Age, n(%)	
< 7 days	139 (92.7)
7-14 days	6 (4)
15-28 days	5 (3.3)
Gestational age, n(%)	
< 38 weeks	41 (27.3)
38-42 weeks	105 (70)
> 42 weeks	4 (2.7)
Birth weight, n(%)	
1,000- < 1,500 g	5 (3.3)
1,500 - < 2,500 g	50 (33.3)
2,500 - 4,000 g	94 (62.7)
> 4,000 g	1 (0.7)
Kramer's scale, n(%)	
I	16 (10.7)
II	24 (16)
III	75 (50)
IV	31 (20.7)
V	4 (2.6)
TSB, n(%)	
> 5 mg/dL	144 (96)
≤ 5 mg/dL	6 (4)
TcB, n(%)	
> 5 mg/dL	145 (96.7)
≤ 5 mg/dL	5 (3.3)

Table 2. Subjects' TSB and TcB concentrations (N=150)

Characteristics	Minimum	Maximum	Mean (SD)
TSB, mg/dL	4.15	21.66	12.32 (3.4)
TcB, mg/dL	4.03	19.50	13.05 (3.5)

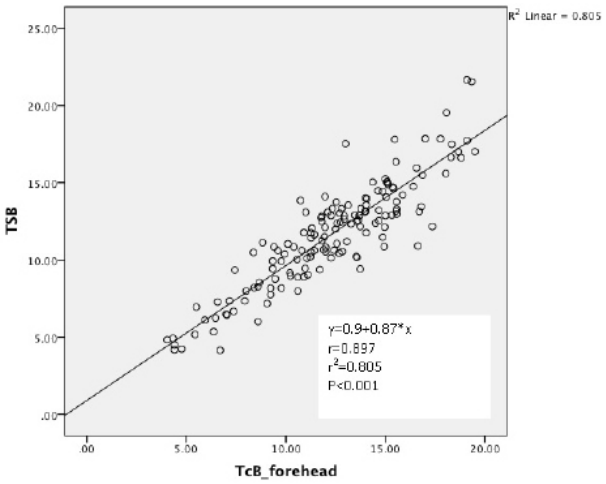

Figure 1. Scatter plot correlation between TSB and TcB measurements

skin. However, the accuracy of these techniques is complicated by variations in skin pigmentation and dermal maturity. Hence, the results of studies in Caucasion infants may not be applicable to the racially heterogeneous Indonesian population.[13,14] The overall correlation between JM-103 measurements and total serum bilirubin estimation was reported to be linear (r = 0.89). The measurements were independent of gestation, race, and ethnicity.[15]

In our study, the correlation coefficient between TSB and TcB (JM-105, at the forehead site) concentrations was high and significant (r = 0.897; P< 0.01), in agreement with studies by Maisels *et al.* in China (r=0.83) and Lamet al.(r=0.83).[16,17] The correlation coefficient does not provide information about clinical significance of the diagnostic test. But we found that as TSB level increased, the difference between values of TcB and TSB increased as well. In addition, the lower the TSB level at which treatment began can lead to frequent blood sampling, a painful procedure with possible complications. The TcB can be used as a screening test to determine the need for TSB measurement.[18]

When comparing TcB and TSB measurements, it is important to remember that the two methods of measurement may be evaluating different physiologic entities. Rubaltelli *et al.* suggested that TcB methods measure the amount of bilirubin that has moved from the serum into the tissue, possibly mimicking the movement of bilirubin across the blood–brain barrier and into brain issue, whereas laboratory-based methods measure only bilirubin that is circulating in the blood. Thus, TcB may actually offer additional information not provided by TSB measurements, although this hypothesis remains to be proven.[19]

The TcB cut-off point obtained from the subjects was > 5 mg/dL, with an AUC of 99.3% (95%CI 97.9 to 100%; P <0.001), sensitivity of 100%, specificity of 83.3%, positive predictive value of 99.3%, and negative predictive value of 100%. A number of studies found different cut-off points. Panburana et al. reported that TcB levels of >12 mg/dL had the

best sensitivity (87.5%) and specificity (96.9%) for predicting TSB.[20]

In conclusion, transcutaneous bilirubin, as assessed by JM-105 has a strongly significant correlation to total serum bilirubin, as measured by chemical laboratory method. The non-invasive TcB measurement (JM-105) is useful as a screening tool to identify those who need serum bilirubin measurements, and can be used in clinical practice to reduce blood sampling. But TcB cannot substitute for total serum bilirubin estimation.

Conflict of interest

None declared.

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Original Article

Lactate profiles of pediatric shock patients in Cipto Mangunkusumo General Hospital 2015: a pilot study

Irena Yuniar

Abstract

Background The 2015 Surviving Sepsis Campaign (SSC) guidelines for management of shock recommend blood lactate to assess the success of resuscitation in shock. However, a study in adults found that 1/3 of septic shock patients had normal lactate levels (alactatemia) and lower mortality rates.

Objective To evaluate lactate profiles, possible factors affecting lactate levels, and mortality outcomes in pediatric shock patients in the emergency room (ER) and pediatric intensive care unit (PICU).

Methods This was a retrospective study on pediatric shock patients aged 1 month to 18 years in the ER or PICU from June 2014 to December 2015. Data were taken from subjects' medical records including lactate levels, examination data required to calculate a PELOD score, and mortality outcomes.

Results Of 223 shock patients evaluated, only 92 cases (41.2%) underwent lactate examinations. Of these, 59 (64.1%) had alactatemia and 33 (35.9%) had hyperlactatemia. A total of 23.7% of the alactatemia group and 36.4% of the hyperlactatemia group died, thus, the initial lactate level was not significantly associated with patient outcomes (P=0.197). The mortality rates of patients with <10% and ≥10% lactate clearance were 31.3% and 17.6%, respectively (P=0.362).

Conclusion In alactatemia patients, lactate level can not be used as a goal for resuscitation. Further study is needed to find a biomarker for assessing the success of pediatric shock resuscitation. Moreover, the clinical relevance of alactatemia is uncertain in pediatric shock patients.**[Paediatr Indones. 2017:57:12-7. doi: 10.14238/pi57.1.2017.12-7]**.

Keywords: *lactate; pediatric; shock; emergency room; pediatric intensive care unit*

Shock remains a problem in the field of pediatrics and is often found in the ER and PICU patients. In developed countries, such as the United States, a reported 37% of pediatric ER patients are in shock.[1] Shock can be classified by etiology, such as hypovolemic shock caused by decreased intravascular volume, cardiogenic shock caused by heart failure as the circulatory pump, distributive shock caused by excessive vasodilatation, endothelial dysfunction, and the loss of vascular tone, as well as obstructive shock caused by obstruction of blood flow to and from the heart.[2] The early management of shock is associated with a lower mortality rate (5.06%, compared to 16.37%of patients with delayed treatment).1 In developing countries, referral delays and limited facilities lead to mortality rates of 47-54.6%, but this number decreases to 11.4-21.8% with early and proper treatment.[3] This finding illustrates the importance of early detection and proper treatment of shock.

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Prior to 2015, physicians followed the 2012 *Surviving Sepsis Campaign* (SSC) guidelines for shock management. The treatments included initial resuscitation with fluid and vasopressor or inotropic drugs, infection control with antibiotics, and other supportive treatments. The 2012 SSC recommended that one of the targets of successful shock resuscitation was lactate clearance value higher than 10% in the first 6 hours of resuscitation.[4] In 2015, revised SSC guidelines added blood lactate examination as a required step in the first three hours.[5] This recommendation was based on the observation of hyperlactatemia often occurring in shock, caused by an increase in lactate production or disruption in lactate clearance.[6] Increased lactate production in shock can be caused by hypoperfusion, which leads to anaerobic metabolism with lactate as the end product.[5] Nevertheless, Hernandez *et al.* found that 1/3 of adult patients with septic shock had alactatemia, and a lower mortality rate.[7] The underlying condition that causes alactatemia is unknown, but it might be due to differences in metabolism in children and adults.[8]

The aim of this study was to evaluate lactate profiles, possible factors affecting lactate levels, and related outcomes in pediatric patients with shock in the ER or PICU.

Methods

This retrospective study was done using the medical records of all pediatric patients aged 1 month to 18 years with shock who were assessed in the ER or treated in the PICU from June 2014 to December 2015. Shock was defined as a clinical condition in which tissue perfusion was unable to meet the demand of tissue metabolism. Types of shock in the subjects' medical records included hypovolemic shock, septic shock, anaphylactic shock, cardiogenic shock, and dengue shock syndrome (DSS).

Patients with incomplete medical records, including data on mortality, blood lactate examination, renal function test, liver function test, and other examinations required to calculate the pediatric logistic organ dysfunction (PELOD), were excluded from the study. The study form was used to record the data from the medical records, which included initials, age, medical record number, dates of admission and discharge, blood lactate level, examination data required to calculate PELOD scores, and mortality outcome.

Data required to calculate PELOD scores included the pediatric Glasgow coma scale (GCS) and pupillary reaction to assess the neurological system, heart rate and blood pressure to assess the cardiovascular system, serum creatinine level to assess renal function, PaO2/FiO2, PaCO2, and the use of mechanical ventilation to assess the respiratory system, leukocyte and thrombocyte counts to assess the hematological system, as well as aspartate transaminase level and prothrombin time (PT) or international normalized ratio (INR) to assess liver function.[9] The values of organ function mentioned above were adjusted to the age of the patient. Patient management was held in accordance with standard procedure for therapy of shock and any medications administered were not recorded in this study.

Blood lactate examinations were not part of the standard protocol for pediatric shock patients in the ER or PICU at Cipto Mangunkusumo Hospital. Subjects' lactate levels were measured in the laboratory using a Nova lactate meter and expressed in mmol/L. Normal lactate level was defined as 0.8-2.5 mmol/L. Alactatemia was defined as lactate level <2.5 mmol/L, while hyperlactatemia was defined as >2.5 mmol/L. Lactate clearance was calculated as the result of the final lactate level (at 6, 12, 24, or 48 hours after a child was diagnosed with shock) minus the initial lactate level, divided by the initial lactate level and multiplied by 100%.

One visit was defined as the time from the day of admission to the day of discharge from the ER or PICU. If a patient had more than one visit during the study period, the second or next visit was counted as a new case. The mortality outcome was defined as died or survived (at the time of discharge). Patients who survived could have been transferred to the ward, or discharged against medical advice from the ER or PICU.

Data was analyzed descriptively and analytically using *SPSS for Windows version 17.0*. Bivariate analysis was used to assess for a correlation between lactate level and PELOD score, renal function test, as well as liver function test. Chi-square was used as the statistical test, unless the criteria for using the test

were not fulfilled, then Fisher's test was done. This study was approved by the Ethics Committee of the University of Indonesia Medical School.

Results

From June 2014 to December 2015, 223 pediatric patients with shock were treated in the ER or PICU of Cipto Mangunkusumo General Hospital. Of the 223 cases, 123 cases (55.4%) had distributive shock, 85 cases (38.1%) had hypovolemic shock, and 15 cases (6.5%) had cardiogenic shock. Of the 123 cases of distributive shock, 113 cases (51% of total shock patients) had septic shock, 9 cases (4%of total shock patients) had dengue shock syndrome (DSS), and 1 case (0.4%of total shock patients) had anaphylactic shock (**Figure 1**).

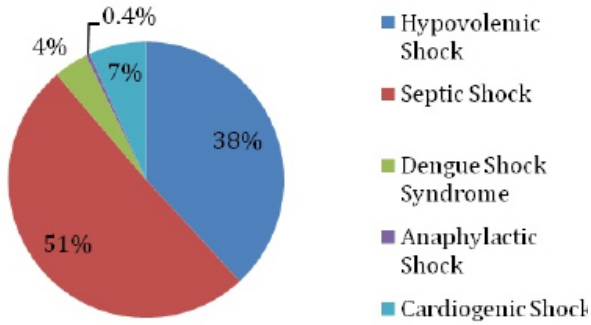

Figure 1. Distribution of types of shock among patients

Of the 92 patients with lactate level data, 23 cases (25%) were hypovolemic, 3 cases (3.3%) were cardiogenic, and 66 cases (71.7%) were distributive shock. Blood lactate was more frequently examined in patients with septic shock (62 cases, 67.4%). There were 59 cases (64.1%) with lactate level < 2.5 mmol/L (alactatemia group) and 33 cases (35.9%) with lactate level ≥ 2.5 mmol/L (hyperlactatemia group). Characteristics of the study subjects are presented in **Table 1**.

The time of lactate examinations varied as follows: 6 patients during the first 0-1 hour, 16 patients (17.4 %) during the next 1-6 hours, 31 patients (33.7%) during the first 6-24 hours, and 39 patients (42.4%) more than 24 hours after shock.

Table 1. Characteristics of subjects with lactate measurements

Characteristics	Total subjects (N=92)	Alactatemia (n=590	Hyperlactatemia (n=33)
Age classification, n(%)	38 (41.3)	24 (40.7)	14 (42.4)
1-12 monoths	37 (40.2)	24 (40.7)	13 (39.4)
13-60 months	5 (5.4)	3 (5.1)	2 (6.1)
60-144 months	12 (13)	8 (13.6)	4 (12.1)
> 144 months			
Gender, n(%)			
Male	51 (55.4)	33 (55.9)	18 (54.5)
Female	41 (44.6)	26 (44.1)	15 (45.5)
Type of shock			
Hypovolemic	23 (25)	15 (25.4)	8 (24.2)
Distributive			
Septic	62 (67.4)	41 (69.5)	21 (63.6)
DSS	4 (4.3)	2 (3.4)	2 (6.1)
Cardiogenic	3 (3.3)	1 (1.7)	2 (6.1)

Serial lactate examinations to ascertain lactate clearance were not routinely performed, as only 45 patients (48.9%) underwent lactate reexamination, 28 patients from the alactatemia group and 17 patients from the hyperlactatemia group. The time of lactate reexamination varied from 1 hour to 48 hours from the first lactate examination. Among 45 patients with lactate reexamination, most of them, 33 of 45 (73.3 %) had lactate reexamination in period of 48 hours. Only 5 of 33 patients (15 %) had lactate reexamination in first 6 hours. Lactate clearance in the first 48 hours is presented in **Table 2**.

Table 2. Lactate clearance from 33 patients with lactate reexamination in 48 hours

Lactate clearance	Alactatemia	Hyperlactatemia	P value
< 10%	12	4	0.049
≥ 10%	7	10	

Most alactatemia cases had lactate clearance of <10% (12/14). In the hyperlactatemia group, more than half (10/14) had a lactate clearance >10%. Significantly more hyperlactatemia subjects had >10% lactate clearance than alactatemia subjects (P=0.049) (**Table 2**).

The initial lactate level was not associated with patient mortality outcomes. Mortality rate was 23.7% in the alactatemia group and 36.4% in the hyperlactatemia group (P = 0.197) (**Table 3**).

Lactate clearance was not significantly associated with mortality outcomes. The group with lactate

clearance <10% had a mortality rate of 31.3%, and the group with lactate clearance >10% had a mortality rate of 17.6% (P=0.362).

Table 3. Initial lactate values and mortality

Lactate clearance			P value
	Survived	Died	
Alactatemia	45	14	0.197
Hyperlactatemia	21	12	

We also calculated PELOD scores and analyzed their relationship to lactate level. Of the 92 shock cases with lactate level data, only 58 patients had thorough organ examinations required to calculate PELOD score. There was no significant correlation between total PELOD score and lactate level in the 58 patients (**Table 4**).

Table 4. PELOD score and lactate level

PELOD score, n(%)	Lactate level		P value
	Alactatemia (n=37)	Hyperlactatemia (n=21)	
< 20	20	10	0.637
≥ 20	17	11	

Lactate is excreted on the liver and kidney. Therefore, we further analyzed for correlations between lactate level to liver and kidney function. Liver function tests were performed on 88 out of 92 shock patients underwent lactate examinations. We found no significant correlation between the liver function and lactate level (P=0.111) (**Table 5**). Furthermore, renal function tests were performed on 85 out of 92 shock patients underwent lactate examinations with no significant correlation between lactate level to kidney function (P=0.067) (**Table 6**).

Table 5. Liver function and lactate level

Liver function, n	Lactate level		P value
	Alactatemia (n=55)	Hyperlactatemia (n=32)	
Normal	37	16	0.111
Reduced	18	16	

Table 6. Kidney function and lactate level

Kidney function, n	Lactate level		P value
	Alactatemia (n=37)	Hyperlactatemia (n=21)	
Normal	43	19	0.067
Reduced	11	12	

Discussion

To date, shock remains to be one of the most frequent problems faced by ER and PICU doctors. In developed countries, sepsis is the most common cause of shock in pediatric patients (49-65%), followed by hypovolemic causes (17 - 31%).[10] During the period of study, septic shock occurred in 51% of our cases, followed by hypovolemic shock in 38.1% cases.

Management of patients with septic shock is generally done according to the 2015 SSC guidelines,[5] which recommend blood lactate examination in the first 3 hours after presentation to assess the success of shock resuscitation. Serum lactate level is used to evaluate tissue oxygenation, since tissue hypoxia in shock patients causes anaerobic metabolism, with lactate as the end product.[11]

This pilot study was done to evaluate lactate profiles of pediatric patients with shock in Cipto Mangkunkusumo General Hospital. To date, blood lactate examination has not been incorporated into the treatment guidelines. However, we found that blood lactate examinations were performed in 92 out of 223 (41.2%) shock cases. Among these 92 patients, 63% had lactate levels < 2.5 mmol/L (alactatemia), and 37% had lactate levels ≥ 2.5 mmol/L (hyperlactatemia). In contrast, in adult sepsis study by Hernandez *et al.* reported that only 1/3 of patients with septic shock had alactatemia, and those alactatemia shock patients had lower mortality rates.7 Moreover, Na S et *al.* found that as few as 9.1%of 512 adult septic patients had alactatatemia.[12] In addition, a multi-center study of 2,424 septic shock patients by Cannonet al. found that 37.6% patients had alactatemia.[13]

The cause of alactatemia remains unclear. In our subjects, no well-defined differences in characteristics were observed between the alactatemia and hyperlactatemia groups (**Table 1**). We also noted no significant difference in mortality rates between the alactatemia and hyperlactatemia group (22.4% vs. 38.2%, respectively, P=0.104). Similarly, a study in pediatric patients aged 1 month – 13 years concluded that initial lactate levels could not be used as a prognostic tool for patient outcomes. Munde et al. found similar lactate levels between patients who died and survived.[14] Another study in children concluded that there was no significant difference between the

initial lactate and lactate levels at the first 6 hours; In addition, those two lactate values did not affect mortality rates.[15]

Lactate clearance above 10% is expected to reduce mortality from shock up to 11%.[16] However, in our study, lactate reexamination was not routinely performed. Lactate reexamination was only performed in 45 patients (48.9%), 28 patients from the alactatemia group and 17 patients from the hyperlactatemia group. Moreover, the time of lactate reexamination varied among patients. Only 5.4% of patients underwent lactate reexamination in the first 6 hours, as recommended by the SCC to evaluate the lactate clearance levels. Most patients (33/45 patients, 73.3%) had lactate reexamination in a period of 48 hours.

The SSC issued an update that lactate examination should be done in the first 3 and 6 hours to assess the success of resuscitation.[5] This statement raised the question as to the role of lactate examination in pediatric patients with shock who were alactatemic since the initial diagnosis. To date, the cause of alactatemia in children with shock remains unclear. Therefore, further study is needed to determine the usefulness of lactate to assess the success of fluid resuscitation.

Lactate clearance in the first 48 hours is shown in **Table 2**. Patients who had lactate clearance of >10% were mostly in the hyperlactatemia group (58.8%), while those with lactate clearance <10% were mostly in the alactatemia group (75%). We also found that lactate clearance was not significantly associated with patient mortality. Patients with lactate clearance <10% had a mortality rate of 31.2% and patients with lactate clearance >10% had a mortality rate of 17.6% (P=0.362). In contrast, Munde *et al.* reported that lactate examination in the sixth hour showed differences in patient outcomes, with a lactate clearance cut-off of 30%, between patients who survived and died (sensitivity 75%, specificity 97%, positive predictive value 90%, and negative predictive value 91.42%).[14] In addition, Keswari *et al.* found that increased initial and 24th hour lactate levels increased the risk of mortality [relative risk (RR) 2.9; 95%CI 1.09 to 7.66; (P=0.029) and RR 4.92; 95%CI 1.77 to 13.65; (P=0.002), respectively]. Lactate clearance <10% in 24 hours also increased the risk of mortality [RR 6.50; 95%CI 2.27 to 18.62; (P=0.001)].15

PELOD is a scoring system to determine the severity of organ damage in pediatric sepsis patients. Saraswati et al. showed that subjects with PELOD scores ≥ 20 had a 39.08 times higher mortality risk than subjects with PELOD score < 20.[17] In our study,of the 92 patients who underwent lactate examinations, only 58 had the complete examination data required to calculate PELOD scores. We found no association between PELOD score and lactate level in shock patients (**Table 4)**, similar to a study by Ismyet al.[18] Therefore, lactate level didn't affect the severity of sepsis marked by PELOD scores.

Lactate is an indirect indicator of sepsis severity in pediatric patients. The cause of alactatemia in children is not known and there may be metabolism differences between pediatric and adult patients.[8] Many factors can affect lactate level and lactate clearance in children. Alactatemia may happen despite an increase in lactate production, if the body has good hepatic and renal lactate clearance. If the liver and renal functions deteriorate, lactate level is predicted to increase.[19] We found that 66.2% of the alactatemia group had reduced kidney function based on serum creatinine level, whereas 52.2% of the hyperlactatemia group had normal kidney function. However, the difference was not significant (P=0.121). Assessment of liver function by alanine aminotransferase (ALT) enzyme revealed normal liver function in 69.0% of patients in the alactatemia group, and 50% of children in the hyperlactatemia group. Therefore, further study on liver and renal function in pediatric shock patients with alactatemia should be done with hypoxia biomarkers.

The use of lactate as a biomarker of the success of resuscitations recommended by the 2015 SSC should be examined further in children. Previous studies found that lactate did not increase in some adult with sepsis (alactatemia).[7,11,12] In this pilot study, we find similar results, as 63%of our pediatric shock patients have alactatemia. Hence we conclude that lactate clearance, particularly in alactatemic conditions, can not be used as a means of assessing the success of fluid resuscitation. Lactate level is not associated with mortality nor with severity of disease. Further study is needed to elucidate the causes of alactatemia in pediatric patients with shock.

Conflict of Interest

None declared.

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Original Article

Domperidone and maternal milk volume in mothers of premature newborns

Tengku Ellya Fazilla, Guslihan Dasa Tjipta, Muhammad Ali, Pertin Sianturi

Abstract

Background Mothers of premature newborns often have difficulty giving adequate breast milk volume to their infants. Domperidone is an antagonist of peripheral dopamine receptors and believed to increase breast milk production. In Indonesia, no study has been done to date on the effect of domperidone on maternal milk production in mothers of premature newborns.

Objective To evaluate the effect of domperidone on milk production in mothers of premature newborns who failed to lactate.

Methods A randomized controlled trial was conducted from July to December 2012 in the Perinatology Unit, Haji Adam Malik Hospital, Medan. Mothers of premature newborns were given lactation counseling for 7 days in order to increase their milk production. Mothers who failed to lactate after that time were enrolled in the study. Fifty subjects were assigned to receive either domperidone or a placebo for 7 days. Milk volume was measured every 2 hours (from 7 am to 9 pm), in the 24 hours before starting therapy, and on the 7*th* and 10*th* days (the 10*th* day being 3 days after stopping therapy).

Results This study involved 25 mothers in the domperidone groups and 25 others in placebo group. After 7 days of therapy, mean breast milk volume was significantly higher in the domperidone group than in the placebo group [181.6 (SD 80.2) *vs.* 72.4 (SD 57.8) mL, respectively; 95%CI of differences 69.36 to 148.93; P=0.0001]. At day 10, breast milk production remained significantly higher in the domperidone group. Furthermore, in the domperidone group, no significant difference in mean breast milk volumes was noted between the 7th and 10th days (P=0.65).

Conclusion In mothers of premature newborns who failed to lactate, domperidone therapy for 7 days causes significantly higher milk production compared to placebo. **[Paediatr Indones. 2017:57:17-22. doi: 10.14238/pi57.1.2017.17-22]**.

Keywords: *domperidone; premature newborn; breast milk*

Low birth weight (LBW) and prematurity are the leading causes of perinatal death in Indonesian hospitals. In 2005, the percentage of live birth infants with low birth weight was 27.9% in Indonesia.[1] Breast milk is the best food for babies and preterm infants. Breastfeeding is beneficial for babies, mothers, families, communities, the environment, and countries. Breastfeeding is expected to decrease the mortality and morbidity of newborn preterm infants.[2] Generally, mothers who give birth to term infants produce sufficient milk in the first week after delivery. However, mothers of preterm babies often have inadequate milk production. Various strategies to increase milk production have been reported, such as relaxation techniques, use of mechanical devices, and medications.[3]

Domperidone is a peripheral dopamine receptor antagonist that is thought to work by blocking the

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inhibitory effects of dopamine-mediated prolactin secretion, thereby increasing milk production.[4,5] There was still a few study on domperidone effects to human milk production. In addition, there has been no study on the effects of domperidone on milk production in Indonesian mothers who gave birth to preterm babies.

We aimed to evaluate milk production in mothers who delivered preterm infants after administering domperidone. We also assessed for possible relationships between milk production and maternal age, gestational age, parity, method of delivery, and educational level.

Methods

A randomized double-blind controlled trial was conducted to assess the effect of domperidone (group A) compared to that of placebo (group B) on milk production in mothers who gave birth to preterm babies. Subjects were recruited from the Perinatology Unit, Haji Adam Malik Hospital, Medan from July to December 2012.

The inclusion criteria were mothers aged 20 to 30 years who gave birth to infants with gestational age < 37 weeks, as well as failure to lactate with little or no improvement in milk production after one week of lactation counseling, along with the baby receiving feeding through a nasogastric tube. Exclusion criteria were mothers who used medications that affect domperidone (e.g., antacids, cimetidin, ranitidine, famotidine, or nizatidine) or medications that interact with domperidone (e.g., haloperidol or lithium), mothers with mastitis, had undergone breast surgery, had heart problems, obesity, diabetes, or twins. Subjects were randomized into domperidone and placebo groups by randomization tables.

Prior to enrollment, mothers underwent ECG examinations to assess heart rhythm, followed by lactation counseling for 7 days in order to maximize breast milk production. If the counseling method failed, then the mothers were eligible to participate in the study. Domperidone was administered orally at a dose of 10 mg, 3 times per day for 7 days. Tablets were crushed, mixed with lactose, then put into capsules. Placebo was lactose powder alone in capsules. All subjects received 21 capsules to be taken 3 times daily for 7 days. A female health worker was employed to supervise the administration of medication and help subjects pump breast milk. Breast milk was collected using a double breast pump (*Medela* ®), for 15 minutes per pump cycle, done daily from 7:00 am to 9:00 pm, every 2 hours. The collected breast milk was measured by researchers and given to the preterm babies. The volume of breast milk counted each day for 1 week. Subjects noted their complaints during treatment, such as dry mouth, headache, insomnia, abdominal pain, diarrhea, nausea, or urinary retention. Breast milk volume was measured at 24 hours prior to the start of medication, 7 days after medication, and day 10 (3 days after stopping the medication). Failure to lactate was defined as decreased breast milk supply (more than 30% of maximum breast milk volume) and/or inability to produce an adequate amount of breast milk to meet the infant's daily nutritional needs.[4]

We used unpaired T-test to compare mean volumes of breast milk in the domperidone and placebo groups. The comparison of mean breast milk volumes on days 7 and 10 in both groups were analyzed by paired T-test. Multivariate analysis was used to determine the relationship between breast milk volume and maternal age, gestational age, number of children, method of delivery, and educational level. Data processing was done with *SPSS 15* software, and results were considered to be significant for P values <0.05 and 95% confidence intervals (CI). This study based on intention to treat analysis.

Subjects provided informed consent for participation. This study was approved by the Ethics Committee of the University of Sumatera Utara Medical School.

Results

At our hospital there were 107 preterm births from July to December 2012, of whom 64 mothers had inadequate breast milk volume. Twelve mothers were excluded because their infants were of gestational age > 37 weeks (4 mothers), they were aged > 30 years (3 mothers), they were obese with BMI > 30 kg/m2 (3 mothers), or they delivered twins (2 mothers). Fifty-two mothers who met the inclusion criteria underwent ECG examination to screen for cardiac arrhythmias; all had normal ECG results. These mothers underwent

a lactation counseling program for 7 days to learn to increase their breast milk production. Two infants died during treatment due to respiratory distress from hyaline membrane disease and sepsis. The remaining 50 mothers completed the lactation counseling program, but failed to lactate. These mothers were randomized into the domperidone or placebo groups, each consisting of 25 mothers. During the study, four mothers dropped out because their infants died, three from the placebo group and one from the domperidone group (**Figure 1**). The infants who died were diagnosed with respiratory distress due to hyaline membrane disease and sepsis.

Table 1 shows the characteristics of subjects. Mean maternal ages in the domperidone and placebo groups were 26 and 25 years, respectively. Mean number of children borne by mother in both groups was one, and mean gestational age in both groups was 31 weeks. More subjects in the domperidone group delivered vaginallly than by caesarean section, but in the placebo group, more subjects underwent caesarean section than vaginal delivery. Most mothers in both groups finished high school (48% and 52%, respectively) and 96% in both groups were housewives. Mean infant birth weights in the domperidone and placebo groups were 1,656 and 1,636 grams, respectively, while mean infant age at hospital admission for both groups was 5 days. Mean breast milk volume prior to therapy was 83.3 (SD 42.99) mL in the domperidone group and 66.6 (SD 49.84) mL in the placebo group.

Differences in breast milk volume between the domperidone and placebo groups on day 7 of treatment and day 10 (3 days after stopping treatment) are shown in **Table 2.** Unpaired T-test revealed that the domperidone group had significantly greater mean breast milk volume than the placebo group on both days (P=0.0001, for day 7 and day 10).

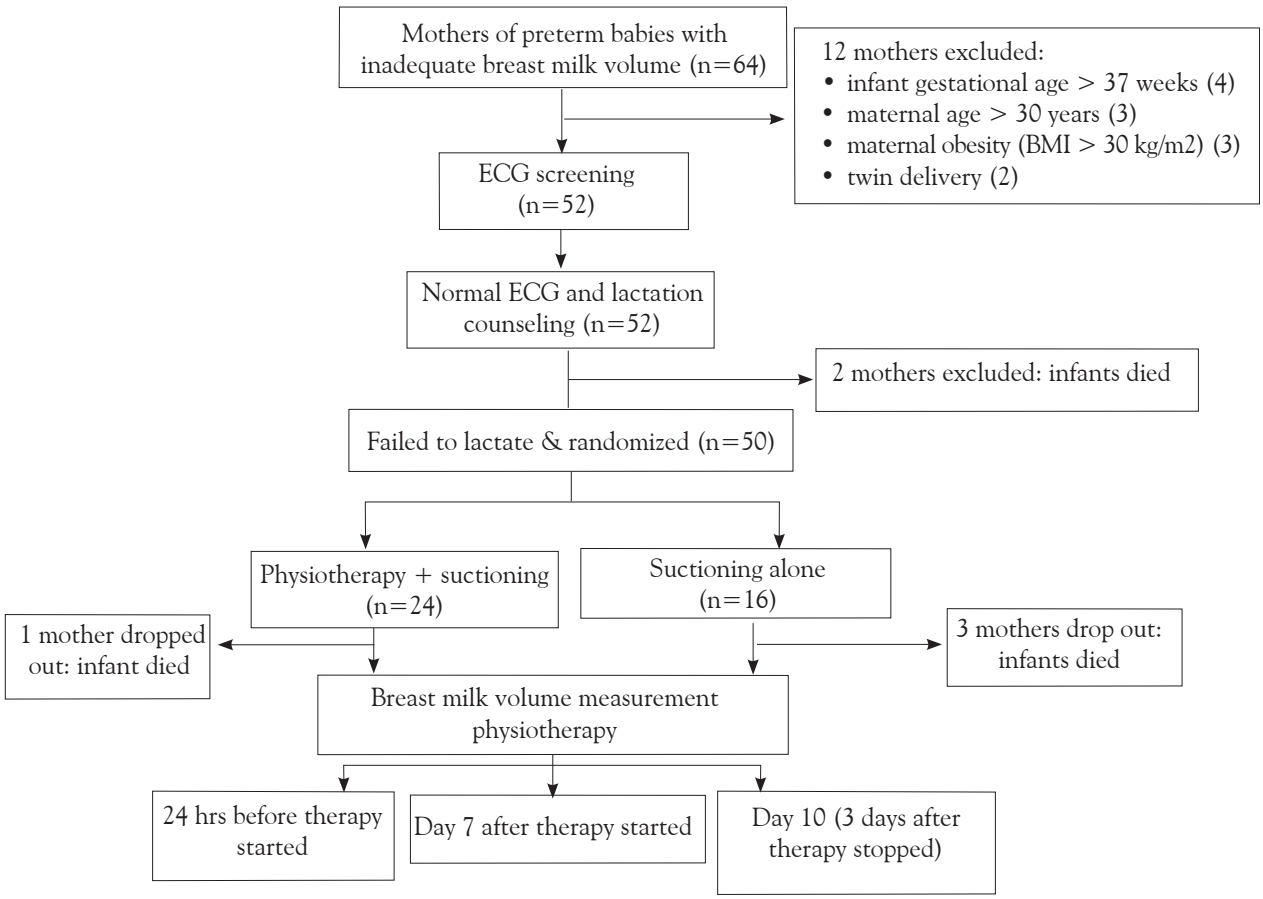

Figure 1. Study profile

Table 1. Baseline characteristics of subjects

Characteristics	Domperidone group (n=25)	Placebo group (n=25)
Mean maternal age (SD), years	26.8 (3.47)	25.7 (3.67)
Mean number of children born by mother (SD)	1.9 (0.83)	1.7 (0.9)
Method of delivery, n		
Vaginal	14	11
Cesarean section	11	14
Maternal education, n		
Primary school	8	7
Junior high school	4	5
Senior high school	12	13
Diploma	1	0
Scholar/master	0	0
Employment, n		
Government	0	0
Private sector	0	0
Enterpreneur	1	1
Farmer/fisherman	0	0
Housewife	24	24
Mean birth weight (SD), grams	1,656.8 (346.2)	1,636.8 (386.75)
Mean infant age (SD), days	5.6 (6.75)	4.8 (4.42)
Mean breast milk volume prior to therapy onset (SD), mL	83.3 (42.99)	66.6 (49.84)

Table 3 shows the comparison of mean breast milk volume on day 7 of treatment and day 10 in the domperidone group. Paired T-test revealed no significant differences in mean breast milk volume ($P>0.05$) between day 7 and day 10. In the placebo group, there were no significant differences in mean breast milk volume before treatment, day 7, and day 10 ($P>0.05$).

Multivariate linear regression analysis of number of children, maternal education, and type of therapy on day 7 of treatment showed that type of therapy could be used to predict breast milk volume on day 7 of treatment (correlation strength 0.623). The equation was breast milk volume = 72.46 + 109.14 (therapy type). Domperidone had a value of 1, while placebo was given a value of 0. ANOVA test revealed $P<0.05$, so we concluded that the equation was appropriate.

Table 3. Comparison of breast milk volume on days 7 and 10 in the domperidone group

Day of treatment	n	Mean breast milk volume (SD), mL	95% CI of differences	P value
Day 7	25	181.6 (80.26)	-8.67 to 13.64	0.65
Day 10	25	179.1 (82.4)		

None of our subjects complained of possible domperidone side effects, such as dry mouth, headache, abdominal pain, or tension in the breasts.

Discussion

In our study, the mean age of mothers who received domperidone was 26 years, with infants of 31 weeks mean gestational age. Characteristics of subjects in this study were similar to those in a 2006 Canadian study. A previous study assessed domperidone effects on breast milk production in subjects aged 28 years who gave birth vaginally to infants of 29 weeks gestation. They had only 16 subjects, whereas our study involved 50 subjects, and they did not assess the method of delivery, although Caesarean section is known to be a risk factor for lactogenesis delay.6 However, our study showed no difference in breast milk volume regarding method of delivery.

We found significantly increased breast milk production in the domperidone group on day 7 of treatment. Mean breast milk volume in the domperidone group was 181.6 (SD 80.2) mL *vs.* 72.4 (SD 57.8) mL in the placebo group (95%CI 69.36 to 148.93; $P < 0.0001$). This finding was consistent with a 2012 systematic review that assessed effects of domperidone on lactation insufficiency in women who gave birth to preterm and full-term infants. The results of three randomized, double-blind studies all showed significant increases in breast milk production after administration of domperidone. Data analysis showed significant increases of 75.36% (95%CI 55.42 to 95.3; $P=0.00001$) in daily breast milk production after taking domperidone compared to placebo. A limitation of this meta-analysis was that the three clinical trials had small

Table 2. Comparison of breast milk volume between the domperidone and placebo groups

Mean breast milk volume (SD), mL	Domperidone group	Placebo group	95% CI of differences	P value
Day of treatment				
Day 7	181.6 (80.26)	72.46 (57.84)	69.36 to 148.93	0.0001
Day 10	179.12 (82.4)	69.32 (51.74)	70.67 to 148.93	0.0001

sample sizes (17, 16, and 45 subjects each). Clinical trials with small sample sizes may be subject to random error.[7] A large, multi-center study was conducted in Toronto in 2012 as a randomized, double-blind clinical trial of 560 mothers who gave birth to preterm babies. This study was still running as of the writing of this paper, as a protocol study.[8]

Domperidone increases breast milk production by working as dopamine receptor antagonist in the striata area, acting to inhibit the anterior pituitary dopamine receptor or the tuberoinfundibular system. Domperidone blocks the inhibitory effects of dopamine-mediated prolactin secretion in the anterior pituitary, resulting in increased serum prolactin levels and thus, breast milk production.[9,10,11] The recommended dose of domperidone as a galactogogue is 10 mg orally, administered 3 times per day for 1 to 2 weeks.[7,12] However, there is no definitive guideline on the domperidone dose that will increase breast milk production. A randomized clinical trial with double-blind design was conducted in Australia on 6 mothers of preterm infants. Mothers received domperidone at doses of 30 and 60 mg/day. Two-thirds of subjects showed significant increases in breast milk production and serum prolactin. The increase in breast milk production occurred when the dose was increased from 30 to 60 mg, although the amount of domperidone in breast milk was quite low and has minimal risk to breastfed babies.[10] Another Canadian study in 2012, compared two doses of domperidone to increase breast milk production and found that an increase of domperidone dose from 10 mg orally 3 times daily to 20 mg increased breast milk production, similar to previous studies.[8] However, Health Canada issued a warning about domperidone doses exceeding 30 mg/day.[13] We used a minimum dose of 10 mg orally 3 times per day as no Indonesian studies have assessed the optimum dose of domperidone to increase breast milk production. Moreover, this dose was chosen to avoid possible side effects, especially arrhythmia.

The optimal duration of domperidone treatment to increase breast milk production is unknown. Previous studies used domperidone for 7, 10, and 14 days as well as 4 weeks.[2,8,14,15] All of these studies showed increased breast milk production after administering domperidone. We administered domperidone for 7 days due to difficulty subject compliance for taking medication and pumping breast milk every 2 hours each day. Before the study began, subjects underwent lactation counseling for 7 days and were asked to pump their breast milk every 2 hours. Hence, we required only 7 days of treatment in order to improve subject adherence to the regimen.

Breast milk volume at day 7 of treatment was not significantly different to that at 3 days after stopping the treatment [181.6 (SD 80.26) mL *vs.* 179.1 (SD 82.4) mL, respectively (P=0.65)]. This finding suggests that breast milk production remained increased 3 days after stopping treatment.

Factors that were assessed for their effect on breast milk volume in this study were number of children borne by mother, maternal education, and type of medication. Multivariate analysis showed that type of medication with a strength of correlation of 0.623 could be used to predict breast milk volume on day 7 of treatment.

Subjects had no complaints about side effects such as dry mouth, headache, abdominal pain, or tension in the breasts. Arrhythmia is a serious side effect that can occur. We assessed subjects for arrhythmias by ECG examination and found no abnormalities in all 50 subjects. With regards to possible side effects, the *Food and Drug Administration* (FDA) in 2004 suggested that breastfeeding mothers not take domperidone due to the risk of cardiac arrhythmias and sudden death. These fates were observed in cancer patients with low potassium levels who received high-dose intravenous domperidone with chemotherapy.16 However, the FDA-issued warning was somewhat of an overreaction, because the subjects who received domperidone had comorbidities, were undergoing chemotherapy, and had severe hypokalemia. In addition, the bioavailability of oral domperidone is only 13 to 17% and peak levels of domperidone at doses of 10 mg orally are only about 1/30 that of administration via the parenteral route.[13]

Limitations of the study were that we did not measure serum prolactin or domperidone levels in breast milk, due to lack of measurement tools and facilities in Haji Adam Malik Hospital. We also did not assess the optimum dose and duration of domperidone administration to maintain breast milk production. Breast milk composition such as protein, glucose, and lipid levels were also not determined in the study.

In conclusion, domperidone significantly increases breast milk production compared to placebo

in mothers who gave birth to preterm babies. In the domperidone group, mean breast milk volume 3 days after stopping treatment is not significantly different from mean breast milk volume at day 7 of treatment. No side effects associated with domperidone treatment is found in our subjects.

Conflict of interest

None declared.

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Original Article

Upper arm circumference measurement for detecting overweight and obesity in children aged 6-7 years

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Abstract

Background Obesity is a worldwide problem and is associated with increased risk of metabolic syndrome. Nutritional status in children has traditionally been determined by body mass index (BMI) scores, but with limitations. Upper arm circumference measurement may be a better predictor of energy, protein, and fat storage, as well as a simpler method for screening overweight and obesity in children.

Objective To determine the diagnostic value of upper arm circumference compared to BMI for detecting overweight and obesity in children aged 6-7 years.

Methods This diagnostic study with a cross-sectional design was performed from September to October 2015 at 16 primary schools in Palembang, Indonesia. We measured the heights, weights, and upper arm circumferences, and calculated BMIs of 2,258 children. Receiver-operator characteristic (ROC) curve analysis was used to find an optimal upper arm circumference cut-off point to detect overweight and obesity. Diagnostic value was calculated by using a 2x2 table analysis.

Results The prevalences of overweight and obesity were 5.8% and 11.7%, respectively. The optimal upper arm circumference cut-off points for detecting overweight in children aged 6-7 years was 185 mm (sensitivity 88.1% and specificity 78.3%), and for obesity was 195 mm (sensitivity 90.15% and specificity 86.65%). Upper arm circumference had a strong correlation with BMI.

Conclusion Upper arm circumference measurement is an accurate method for–distinguishing between normoweight, overweight, and obesity in children aged 6-7 years. **[Paediatr Indones. 2017;57:23-9. doi: 10.14238/pi 57.1.2017.23-9]**.

Keywords: *childhood obesity; upper arm circumference; children aged 6-7 years*

Obesity is a worldwide problem. Pediatric obesity is associated with increased risk of metabolic syndrome in adulthood. The prevalence of obesity is increasing in both developed and developing countries. The prevalence of obesity has increased from 5% in 1963-1970 to 17% in 2003-2004.[1] The 2013 Indonesian Health Research Survey (*Riskesdas*) reported that the prevalence of overweight in children 5-12 years old were 10,8% and obesity were 8,8%.[2]

Obesity is defined as a disorder or a disease characterized by the accumulation of excessive body fat tissue. Using BMI charts (CDC 2000), a BMI ≥ 85 - <95 percentile is classified as overweight, and BMI ≥ 95th percentile is classified as obese.[3,4] Obesity occurs because of an imbalance between energy intake and energy output (expenditure).[5] Most energy homeostasis disorders are caused by idiopathic factors (primary and nutritional obesity), while fewer are caused by endogenous factors (secondary or non-nutritional obesity, caused by hormonal disorders,

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syndromes, or genetic defects).[6] The clinical manifestations of obesity are a rounded face, chubby cheeks, double chin, chest with enlarged breast tissue, and an abdominal wall with folds. Management of obesity consists of several stages which include prevention, structured weight management, and comprehensive multidisciplinary intervention.[3] Body fat content can be measured by underwater weighing examination (hydro-densitometry), magnetic resonance imaging (MRI), computerized tomography (CT), dual-energy X-ray absorptiometry (DEXA), or bioelectrical impedance analysis (BIA). Anthropometric examinations are done by measuring body weight, height, skin fold thickness, abdominal circumference, or upper arm circumference and comparing the results to standardized growth charts for children of similar age and sex.[7]

Determining children's nutritional status generally refers to BMI percentile curve measurements, according to age and sex. However, BMI has several drawbacks including not distinguishing between fat and non-fat mass, or between total body fat and body fat distribution.[8,9] The measurement of BMI requires height and weight scales, as well as the BMI reference charts.[8] An alternative method for diagnosing overweight and obesity is the upper arm circumference measurement. Upper arm circumference can be used to measure growth, protein and energy reserves, as well as to provide information about body fat mass.[10,11] It can be used as a reference because the upper arm is, in theory, cylindrical with subcutaneous fat evenly distributed around the middle upper arm muscles.[12,13] Upper arm circumference measurements also have the advantages of being easier and less expensive, as only a measuring tape and reference tables according to age and gender are required. As such, the measurements can be easily done in community and health facilities.[3, 14] Upper arm circumference based on age can be used to assess nutritional status of children who are sick or have abnormalities in the legs or spine. It is also relatively less influenced by edema and ascites.[15,16]

Past studies have reported upper arm circumference cut-off points,[17] but BMIs and eating habits differ among ethnic groups, necessitating reference values for nutritional status specific to a particular developing country, such as Indonesia. We chose to include subjects aged 6-7 years because adiposity rebound tends to occur at that age period, when rapid body weight increases, may affect the prevalence of obesity in adolescence and adulthood.[4, 18]

We aimed to assess upper arm circumferences of 6-7-year-olds and compare them to their BMI measurements, in order to determine upper arm circumference cut-off points for detecting overweight and obesity in children aged 6-7 years.

Methods

This diagnostic study with a cross-sectional design was done in September to October 2015. Data are presented in tabular form and ROC curve analysis. Subjects were children aged 6-7 years from 16 primary schools in Palembang, who were recruited by cluster sampling determined by the topography of Palembang which devided into area ulu and ilir. Three until four shcool in a subdistrict were chosen to included in this reasearched. Children with severe deformity of vertebrae, upper arm, or lower extremity, Down or Turner syndrome, received long-term steroid treatment, or who were uncooperative during the examination were excluded. Subjects indicated they were willing to join the study and their parents provided informed consent. The study was approved by the Committee for Medical Research Ethics of University of Sriwijaya Faculty of Medicine.

We measured subjects' heights, weights, upper arm circumferences, and waist circumferences, as well as calculated their BMIs. Data on parental education, job, and income were collected by questionnaire The researchers and five trained assistants used measuring tools that had been calibrated for accuracy, including weight scales, stature meters, and measuring tapes SECA brand. We used the 2000 CDC BMI reference standard curves.[3] Subjects were classified as normoweight for BMI $< 85^{th}$ percentile, overweight for BMI $\geq 85^{th}$ - $<95^{th}$ percentile, and as obese for BMI $\geq 95^{th}$ percentile.

Data were analyzed by *SPSS for Windows 19.00* (*SPSS Inc*) software. The ROC curve analysis was used to determine optimal upper arm circumference cut-off points to detect overweight and obesity and to distinguish between them. Diagnostic values were calculated by a 2x2 table analysis.

We used ROC curve analysis to determine the validity of upper arm circumference for detecting

overweight in children aged 6-7 years compared to BMI. The area under the curve (AUC) and the coordinates were used to determine the optimal upper arm circumference cut-off values for assessing nutritional status. The analysis was done in two stages: first, by including normoweight, overweight, and obese; and second, by normoweight and overweight only, in order to determine the diagnostic upper arm circumference value to distinguish between normoweight and overweight.

Results

From September to October 2015, anthropometric measurements were taken on 2,258 children who met the inclusion criteria. Using BMI reference standards, 131 (5.8%) were classifed as overweight, 264 (11.7%) were obese, 581 (25.9%) were underweight, and 1,282 (56.6%) were normoweight. No subjects dropped out of the study. The ratio of boys to girls was 1.05:1.

The upper arm circumference AUC value for males and females to distinguish normoweight from overweight and obese was 89.8% (95%CI 87.7 to 91.9%). The AUC value for males was 89.8% (95%CI 87 to 92%), and for females was 88.1% (95%CI 84 to 92%; P<0.001). The optimal upper arm circumference cut-off point for detecting overweight in children was 185 mm. The table analysis and diagnostic values of upper arm circumference for detecting overweight compared to BMI are shown in **Table 1**.

An upper arm circumference cut-off point of 185mm for distinguishing normoweight from overweight and obese in children as compared to BMI had a sensitivity of 88.1%, specificity of 78.3%, positive predictive value of 55.6%, negative predictive value 95.5%, positive likelihood ratio 4.05), negative likelihood ratio of 0.15, and accuracy of 80.6%. For boys alone, this cut-off point had a sensitivity of 89.2%, specificity of 78.3%, positive predictive value of 59.4%, negative predictive value of 96.8%, positive likelihood ratio of 4.1, negative likelihood ratio of 0.15, and accuracy of 81.2%. For girls alone, this cut-off point had a sensitivity of 86.6%, specificity of 78.3%, positive predictive value of 51.2%, negative predictive value of 95.7%, positive likelihood ratio of 3.99, negative likelihood ratio of 0.17, and accuracy of 66.2%.

With the same cut-off point of 185mm, the AUC value of upper arm circumference to distinguish between normoweight and overweight children was 82.8% (95%CI 78.6 to 87%; P < 0.001). The AUC value of upper arm circumference for males was 80.5% (95%CI 74.3 to 86.7%; P<0.001), while that for females was 84.8% (95%CI 79.2 to 90.4%; P<0.001). The 2x2 table analysis results and diagnostic value of upper arm circumference for detecting overweight in children as compared to BMI is shown in **Table 2**.

An upper arm circumference cut-off point of

Table 1. Diagnostic value of upper arm circumference for detecting overweight and obesity compared to BMI (cut-off point ≥185 mm)

Criteria	Upper arm circumference	BMI		Total
		>P_{85}	P_{5-85}	
Male & female,* n	≥185 mm (overweight + obese)	348	278	626
	<185 mm (normoweight)	47	1,004	1,051
	Total	395	1,282	1,677
Male,** n	≥185 mm (overweight + obese)	199	136	335
	<185 mm (normoweight)	24	491	515
	Total	223	627	850
Female,*** n	≥185 mm (overweight + obese)	149	142	291
	<185 mm (normoweight)	23	513	536
	Total	172	655	827

*(Sen 88.1%; Spec 78.3%; PPV 55.6%; NPV 95.5%; LR^+ 4.05; LR^- 0.15 accuracy 80.6%)

**(Sen 89.2%; Spec 78.3%; PPV 59.4%; NPV 96.8%; LR^+4.1; LR^- 0.15 accuracy 81.2%)

***(Sen 86.6%; Spec 78.3%; PPV 51.2% NPV 95.7%; LR^+3.99; LR^- 0.17, accuracy 66.2%)

Sen=sensitivity; Spec=specivicity; PPV=positive predictive value; NPV=negative predictive value; CR+=positive likelihood ratio; LR-=negative likelihood ratiO

Table 2. Diagnostic value of upper arm circumference for detecting overweight in children aged 6-7 years (cut-off point ≥185 mm)

Criteria	Upper arm circumference	BMI		Total
		P_{85-P95}	P_{5-85}	
Male and female,* n	≥ 185 mm (overweight)	103	278	381
	< 185 mm (normoweight)	28	1,004	1,032
	Total	131	1,282	1,413
Male,** n	≥ 185 mm (overweight)	44	136	180
	< 185 mm (normoweight)	16	491	507
	Total	60	627	687
Female,*** n	≥ 185 mm (overweight)	59	142	201
	< 185 mm (normoweight)	12	513	525
	Total	71	655	726

*(Sen 78.6%; Spec 78.3%; PPV 27%; NPV 97.2%; LR^+ 3.46; LR^- 0.28 accuracy 78.3%)
**(Sen 73.3%; Spec 78.3%; PPV 24.4%; NPV 96.8%; LR^+ 3.22; LR^- 0.35 accuracy 77.8%)
***(Sen 83%; Spec 78.3%; PPV 41.5%; NPV 97.7%; LR^+ 3.8; LR^- 0.21 accuracy 78.8%)

185 mm for distinguishing between normoweight and overweight in children aged 6-7 years compared to BMI had sensitivity of 78.3%, specificity of 78.3%, positive predictive value of 27%, negative predictive value of 97.2%, positive likelihood ratio of 3.46, negative likelihood ratio of 0.28, and accuracy of 78.3%. For boys alone, this cut-off point had sensitivity of 73.3%, specificity of 78.3%, positive predictive value of 24.4%, negative predictive value of 96.8%, positive likelihood ratio of 3.22, negative likelihood ratio of 0.36, and accuracy of 77.8%. For girls alone, this cut-off point had sensitivity of 83%, specificity of 78.3%, positive predictive value of 41.5%, negative predictive value of 97.7%, positive likelihood ratio of 3.8, negative likelihood ratio of 0.21, and accuracy of 78.8%.

ROC analysis revealed that the optimal upper arm circumference cut-off point to distinguish obesity from overweight and normoweight was 195 mm. The AUC value of upper arm circumference for detecting obesity in children aged 6-7 years was 92.9% (95%CI 90.9 to 94.9%; P < 0.001) (**Figure 1**). The AUC value of upper arm circumference for boys was 94.8% (95%CI 92.7 to 96.8%; P < 0.001), while that for detecting obesity in girls was 90% (95%CI 86.1 to 94%; P < 0.001). The table analysis results and diagnostic value of upper arm circumference for detecting obesity in children compared to BMI is shown in **Table 3**.

An upper arm circumference cut-off point of 195 mm for detecting obesity in children aged 6-7 years compared to BMI had a sensitivity of 90.2%, specificity of 86.5%, positive predictive value of 47.1%, negative predictive value of 98.5%, positive likelihood ratio of 6.28, negative likelihood ratio of 0.13, and accuracy of 87%. For boys alone, this cut-off point had sensitivity of 93.25%, specificity of 86.9%, positive predictive value of 53.9%, negative predictive value of 98.7%, positive likelihood ratio of 6.64, negative likelihood ratio of 0.77, and accuracy of 87.8%. For girls alone, this cut-off point had sensitivity of 85.1%, specificity

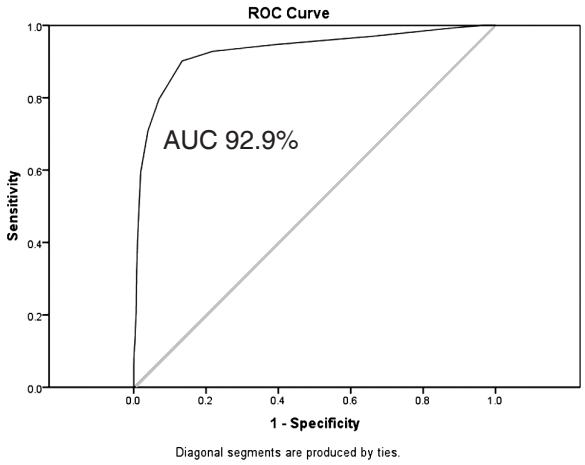

Figure 1. ROC curve of upper arm circumference compared to BMI for distinguishing obesity in children aged 6-7 years

of 86.2%, positive predictive value of 38.6%, negative predictive value of 98.28%, positive likelihood ratio of 6.15, negative likelihood ratio of 0.17, and accuracy of 86.1%.

Pearson's correlation test revealed that the upper arm circumference had a strong correlation with weight (r=0.78; P=0.000), height (r=0.44; P=0.000), and BMI (r=0.73; P=0.000).

Table 3. Diagnostic value of upper arm circumference for detecting obesity in children aged 6-7 years (cut-off point ≥195 mm)

Criteria	Upper arm circumference	BMI		Total
		≥ P_{95}	< P_{95}	
Male & female,* n	≥ 195mm (obese)	238	267	505
	< 195mm (non-obese)	26	1,727	1,753
	Total	264	1,994	2,258
Male,** n	≥ 195mm (obese)	152	130	282
	< 195mm (non-obese)	11	866	877
	Total	163	996	1,159
Female,*** n	≥ 195mm (obese)	86	137	223
	< 195mm (non-obese)	15	861	876
	Total	101	998	1,099

*(Sen 90.15%; Spec 86.65%; PPV 47.1%; NPV 98.5%; LR^+ 6.28; LR^- 0.13 accuracy 87%)
**(Sen 93.25%; Spec 86.9%; PPV 53.9%; NPV 98.7%; LR^+ 6.64; LR^- 0.77 accuracy 87.8%)
*** (Sen 85.1%; Spec 86.2%; PPV 38.6%; NPV 98.28%; LR^+ 6.15; LR^- 0.17; accuracy 86.1%)

Discussion

This diagnostic study was done to determine the accuracy of diagnostic upper arm circumference values compared to body mass index for detecting overweight and obesity in a pediatric population. The male to female ratio of children aged 6-7 years was 1.05:1.

In Indonesia, children's nutritional status is generally determined by BMI curves (CDC 2000), according to age and sex. Children with BMI in the 85th to < 95th percentile are considered to be overweight, and those with BMI ≥ 95th percentile are considered to be obese.[3] In our study, the prevalence of overweight and obesity were 5.8% and 11.7%, respectively. This finding is consistent with the 2013 *Riskesdas* prevalence of obesity in school-aged children of (11.9%).[2] The obesity prevalence of boys in our study (14%) was higher than that of school-aged boys in Indonesia (10.7%) (2010 *Riskesdas*), but lower than that of boys in South Sumatra (16.0%) (2007 *Riskesdas*).[19,20] The obesity prevalence of girls in our study (9.2%) was higher than that of school-aged girls in Indonesia (7.7%) (2010 Riskesdas), but lower than that of school-aged girls in South Sumatra (11.0%) (2007 Riskesdas).[19,20] Furthermore, we found that the prevalence of obesity in boys was higher than in girls, consistent with the 2007 and 2010 *Riskesdas* results.[19,20]

Human activity that involves repetitive muscle movement in an upper extremity can increase muscle mass in an asymmetric fashion. Brown and Wolpert reported that upper extremities of individuals may be asymmetric and significantly different in circumference.[21] However, we found no difference between right and left upper arm circumference measurements in children because the stress markers of handedness which are influenced by repetitive movement of the dominant hand in the children aged 6-7 years has not yet happened.[21]

The mean BMI of boys aged 6-7 years in our study was 15.66 kg/m^2, which was lower than boys in the UK (15.8 kg/m^2), Germany (15.8 kg/m^2), China (16.5 kg/m^2), and Qatar (17.7 kg/m^2). The mean BMI of girls aged 6-7 years was 15.14 kg/m^2, which was similar to girls in Qatar (15.1 kg/m^2), but lower than girls in the UK (15.4 kg/m^2), Germany (15.6 kg/m^2), and China (15.9 kg/m^2).[22-25]

In our study, the mean body weights were 21.29 kg in boys and 20.16 kg in girls. Mean heights were 116 cm in boys and 114.85 cm in girls. The mean abdominal circumferences were 55.86 cm in boys and 54.87 cm in girls.

Upper arm circumference can be used to measure growth, as an indicator of protein and energy reserves, as well as provide information on body fat levels.[3] The upper arm circumference cut-off point was the same between our male and female subjects. In contrast, a South African study found different cut-off points for obesity in boys and girls aged 5-9 years (192 mm *vs.* 184 mm, respectively). A Nigerian study showed significantly higher fat mass and upper arm circumference in girls than in boys, in children aged 5-15 years. This observation may be due to total body fat increases to prepare for a future growth spurt during adolescence. This increased total body fat and puberty occurs in girls earlier than in boys (19% female and 14% male). In the early teen years, boys have more muscle mass than girls.[26] Since our sample population was 6-7-year-olds, we found no difference in cut-off point between males and females.

The mean upper arm circumferences were 182 mm in boys and 179 mm in girls. These values were higher than the mean upper arm circumference of 171 mm (males and females) reported in a Turkish study.[7] Our 185 mm cut-off point to distinguish normoweight from overweight was also higher than their cut-off points of 181 mm for boys and 179 mm for girls. However, all the values were lower than the US 90th percentile of health and nutrition of 209 mm for boys and 204 mm for girls. Our upper arm circumference cut-off point to distinguish obese from non-obese was 195 mm, which was lower than those of US children (226 mm for boys and 211 mm for the girls), but higher than the Turkish study (182 mm in boys and 180 mm in girls). This difference may be due to the small sample size in the Turkish study of 124 boys and 126 girls. [17]We found that the sensitivity of upper arm circumference in distinguishing normoweight from overweight was higher (88.1%) when the obese subjects were included in the diagnostics measurement, likely due to fewer overweight subjects in the study population. The positive predictive values in this study were lower than the negative predictive values, and were as follows: for distinguishing normoweight from overweight and obese: PPV 55.6% and NPV 95.5%; for distinguishing normoweight from overweight: PPV 27% and NPV 97.2%; and for distinguishing obese from non-obese: PPV 47.13% and NPV 98.5%. These findings may have been due to the smaller size of the overweight and obese sample of the population. Therefore, further research is recommended using a sample population of overweight and obese children with its own sample calculation.

The upper arm circumference diagnostic value compared to BMI of each cut-off point in this study was quite high, in addition to the significant correlations between upper arm circumference and BMI. As such, upper arm circumference can be used to predict the presence of overweight and obesity in children aged 6-7 years.

The gold or reference standards for assessing nutritional status are the 2000 CDC BMI curves.[3] Although the Committee for Nutrition and Metabolism of the Indonesian Pediatrics Association recommends using the 2000 CDC BMI standard for children over 5 years of age, it is a limitation of our study in that the reference standard is based on data from children in the United States, which do not necessarily correspond to those of children in Indonesia, as BMI is strongly influenced by age, gender, and race.[27,28] Further study conducted in all age groups of children and adolescents is required in order to compare upper arm circumference data to BMI for each age group.

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Conflict of interest

None declared.

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Original Article

Comparison of SpO2/FiO2 and PaO2/FiO2 ratios as markers of acute lung injury

Dewi Shandi Laila, Chairul Yoel, Hakimi, Munar Lubis

Abstract

Background One of the diagnostic criteria for acute lung injury (ALI) is the PaO_2/FiO_2 (P/F) ratio. This measurement is obtained by blood gas analysis, which involves an invasive procedure (arterial blood draw). In order to reduce invasive procedures on critically ill patients, an alternative non-invasive marker for ALI is needed. The SpO_2/FiO_2 (S/F) ratio attained by pulse oximetry may be a suitable alternative.

Objective To investigate for a correlation between S/F ratio and P/F ratio, in order to find an alternative non-invasive marker of ALI.

Methods A cross-sectional study was conducted in the pediatric intensive care unit (PICU) at Haji Adam Malik Hospital, Medan from August 2012 to June 2013. Subjects (children aged 1 month – 18 years) underwent blood gas analysis when their pulse oximetry showed saturation of 80-97% within 24 hours of ventilator use. We measured PaO_2, SpO_2, and FiO_2 and calculated S/F and P/F ratios. Data were analyzed by Spearman's correlation and linear regression tests.

Results Of 69 PICU patients, 39 children fulfilled the criteria for ALI. The S/F ratio and P/F ratio had a weak correlation (r=0.2; P=0.18). The linear regression equation was S/F ratio = 129.67 + 0.11 (P/F), with S/F ratio values of 162.67 and 151.67 correlating to P/F ratio values of 300 and 200, respectively.

Conclusion The S/F ratio has a weak correlation with P/F ratio for ALI in children. **[Paediatr Indones. 2017;57:30-4. doi: 10.14238/pi 57.1.2017.30-4]**.

Keywords: *acute lung injury; children; S/F ratio; P/F ratio*

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) continue to be significant causes of morbidity and mortality for patients admitted to the PICU.[1] A US study reported that the incidence of pediatric ALI was 12.8 cases per 100,000 person-years, with a hospital mortality rate of 18%. The population incidence rates was lower than that of adult acute lung injury, with 78.9 cases per 100,000 person-years and mortality rates up to 38.5%.[2]

The ALI and ARDS are disorders of pulmonary inflammation, characterized by hypoxemia and respiratory failure.[3] They have been defined as acute hypoxic respiratory failure resulting from pulmonary or extra-pulmonary causes, except cardiogenic factors.[4] ARDS has more severe clinical findings than ALI. All patients with ARDS have ALI, but not all patients with ALI have ARDS.[5] A prospective study in Beijing reported that the survival rate was higher in patients

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with pulmonary disease origin (69.2%) than in the those with extrapulmonary disease origin (33.3%).[4]

In 1994, the American-European Consensus Conference (AECC) defined ALI/ARDS by the criterion of PaO_2 /FIO_2 (P/F) ratio, with ARDS, being the more severe form of ALI.[5] However, obtaining the P/F ratio requires arterial blood gas sampling.[6] In order to reduce invasive procedures in critically ill children, a non-invasive alternative marker for P/F ratio is needed. Routine use of pulse oximetry in most PICUs can minimize the use of blood gas analysis.[6] Generally, PaO_2 changes have correlated to pulse oximetric saturation (SpO_2) changes, in defining hypoxemic grade.[7] A prospective study in children in Los Angeles demonstrated that the SpO_2/FiO_2 (S/F) ratio had a strong correlatation to the P/F ratio, and could be used to identify ALI and ARDS.[8]

The aim of our study was to investigate for a correlation between S/F ratio and P/F ratio, with the goal of identifying an alternative non-invasive marker of ALI.

Methods

This cross-sectional study was conducted in the PICU, Haji Adam Malik Hospital, Medan from August 2012 to June 2013. Subjects were children aged 1 month to 18 years using ventilators. Subjects were collected by consecutive sampling. Specimens for blood gas analysis were taken when the patient's pulse oximetry showed a saturation of 80-97%, within 24 hours of ventilator use. Measurements were excluded if the SpO_2 values were not between 80% and 97%, or if the patient had a diagnosis of congenital heart disease (CHD), arrhythmia, dysrhythmia, shock, or hypothermia. SpO_2 values higher than 97% were excluded because the oxyhemoglobin dissociation curve showed no changes at >97%. This study was approved by the Medical Ethics Committee of the University of Sumatera Utara Faculty of Medicine.

Data were processed and analyzed with *SPSS version 17*, Spearman's correlation and linear regression tests were used for statistical analyses, with 95% confidence intervals.

Results

Of 69 patients who used ventilators during the study period, a total of 39 patients met the inclusion criteria. Thirty patients were excluded. Six patients had CHD and 24 other did not met ALI criterias. Data were

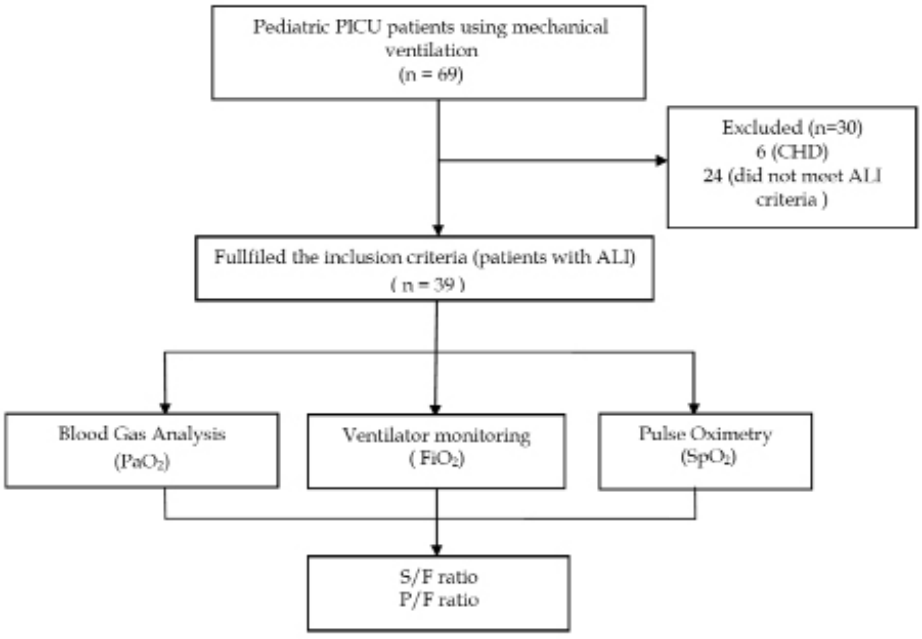

Figure 1. Study profile

collected during the first 24 hours of ventilator use. Blood specimens were used for blood gas analysis to assess PaO_2. At the same time, SpO_2 data from pulse oximetry and FiO_2, as seen from the ventilator readings, were recorded. The study profile is shown in **Figure 1**.

Basic characteristics of subjects are shown in **Table 1**. Subjects were predominantly male, had a mean age of 57 months, mean weight of 18.37 kg, and mean height of 98.01 cm.

Most subjects had bronchopneumonia on chest radiograph results and sepsis was the most common diagnosis in patients with ALI (**Table 1**).

Subjects' mean FiO2 was 60.76. Their mean PaO_2 and SpO_2 were 155.63 mmHg and 94.89%, respectively. The mean P/F ratio was 256.41 and mean S/F ratio was 159.00 (**Table 2**).

Table 1. Characteristics of subjects

Characteristics	(N = 39)
Mean age (range), months	57 (1-204)
Sex, n (%)	
Male	23 (59)
Female	16 (41)
Mean body weight (range), kg	18 (4-65)
Mean body height (range), cm	98 (45-150)
Mean heart rate (range), times/min	105 (76-170)
Mean respiratory rate (range), times/min	24 (14-50)
Mean temperature (range), °C	36.91 (35.8-37.6)
Mean blood pressure (range), mmHg	
Systolic	94.56 (70-140)
Diastolic	55.18 (40-90)
Radiography, n(%)	
Bronchopneumonia	26 (66.66)
Pleural effusion	6 (15.38)
Others	7 (17.94)
Diagnosis at ALI onset, n(%)	
Pneumonia	10 (25.64)
Sepsis	21 (53.84)
Multiple trauma	7 (17.94)
Drowning	1 (2.56)

Since the data were not normally distributed, Spearman's correlation was used to assess for a correlation between S/F ratio and P/F ratio (r=0.215; P=0.18).

Table 2. Oxygen parameters measured

Parameters	(N = 39)
Mean FiO2 (range)	60.76 (40 - 80)
Mean PaO2 (range), mmHg	155.63 (84.2 – 210.5)
Mean SpO_2 (range), %	94.89 (89 - 97)
Mean P/F ratio (range)	256.41 (206 - 300)
Mean S/F ratio (range)	159.00 (106 - 245)

Table 3. Spearman's correlation analysis of S/F ratio and P/F ratio

		S/F ratio
P/F ratio	r	0.215
	P	0.189
	N	39

Figure 2 shows no significant correlation (P>0.05) between P/F and S/F ratios, although S/F generally increased when P/F increased . The R^2 value of 0.9% indicates that the S/F ratio contributed to the P/F ratio by 0.9%, and the remaining 99.1% was determined by other variables.

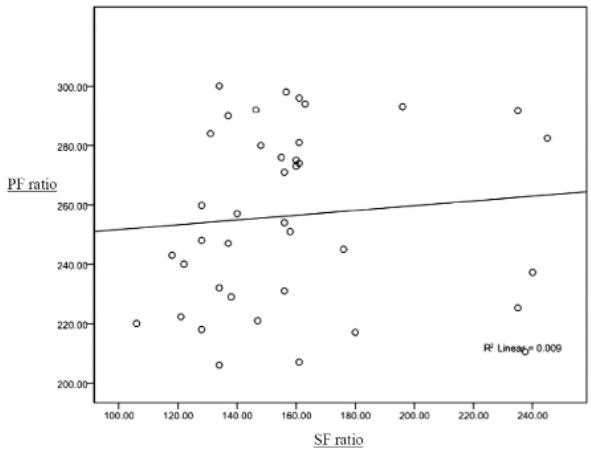

Figure 2. Scatterplot of P/F ratio and S/F ratio

Using the constant values of (a) 129.67 and (b) 0.11, we determined the regression linear equation to be: S/F ratio = 129.67 + 0.11 (P/F ratio). The S/F ratio increased by 0.11 for every 1 point increase in P/F ratio. The S/F ratios of 162.27 and 151.67 correlated with the P/F ratios of 300 and 200, respectively (**Table 4**).

Table 4. Linear regression analysis of S/F ratio and P/F ratio

Model	Correlation coefficient	P value
Constant	129.67	0.010
P/F ratio	0.11	0.545

Discussion

In the 39 children who met criteria for ALI, the most common underlying diseases were sepsis (53.84%) and

pneumonia (25.64%). Similarly, a prospective study in Beijing reported that the most common causes of ALI were pneumonia (52%), sepsis (36%), multiple trauma (9%), lung contusion (5%), and drowning (2%).[4]

The result of this study showed weak correlation between S/F and P/F ratio and was not statistically significant (r = 0.2). A study in Los Angeles compared S/F ratio and P/F ratio in 383 children who suffered ALI/ARDS. From the total of 1,298 blood gas analysis results, the authors reported a moderate correlation (r=0.47).[6] The same ARDS Network study in 672 adult patients who suffered ALI/ARDS from the total of 672 blood gas analysis results showed a strong correlation (r=0.8).[8]

Non-invasive monitoring with pulse oximetry is routinely used in the emergency room and ICU. The measurement of SpO_2 with pulse oximetry may be a tool to predict the PaO_2 value, without using blood gas analysis. However, race, placement of the oximetry sensor, underlying disease, and methemoglobin can diminish the accuracy of the SpO_2 reading.[8]

We found the mean values of PaO_2 and SpO_2 to be 155 mmHg and 95%, respectively. Although the PaO_2 value was above 100 mmHg, the SpO_2 value remained in the range of 88-98%. The value of PaO_2 can increase above 100 mmHg, while SpO_2 is limited to 100%. Since many subjects had PaO_2 values above 100 mmHg, the oxyhemoglobin dissociation curve might not be applicable to our study. The relationship between PaO_2 and SpO_2 in the oxyhemoglobin dissociation curve depicted with two lines. The first line describes the relationship between SpO_2 and PaO_2, with the PaO_2 value in the range of 0 – 60 mmHg. Minor changes in PaO_2 will lead to major changes in SpO_2. The other line describes the relationship between SpO_2 and PaO_2, with the value of PaO_2 above 60 mmHg, forming an almost straight line. As such, major changes in PaO_2 will lead to minor changes in SpO_2.[7]

A prospective, multicenter study in the United States compared SpO_2 and PaO_2 in 137 ALI patients with 1,116 blood gas analysis results. The mean PaO_2 and SpO_2 values were 70 mmHg and 95%, respectively. These values indicated a strong correlation between S/F ratio and P/F ratio.[9] Another study in California showed the mean PaO_2 and SpO_2 values of 69 mmHg and 94%, respectively.[6] These two studies showed good results and in accordance with the principle of oxyhemoglobin dissociation curve.

The projected equation from this study was S/F ratio = 129.67 + 0.11 (P / F ratio). Using this formula, the S/F ratio is expected to increase by 0.11 for every 1 point increase in P/F ratio. The S/F ratio of 162.67 and 151.67 correlated with P/F ratio of 300 and 200, respectively. Another study compared S/F ratio with P/F ratio to predict ALI/ARDS in children. Rice *et al*. reported the equation S/F = 76 + 0.62 (P/F), where S/F ratio values of 263 and 201 correlated with P/F ratio values of 300 and 200, respectively.[8]

In Berlin (2012), improved criteria for ARDS was announced as "*The Berlin Definition*" according to the studies and data in adults. The validity of the definition for use in children was considered quite good, especially for the severe category of ARDS.[10,11] The ALI criteria in this study were based on "*The Berlin Definition,*" including mild ARDS criteria. Further study on the relationship of S/F and P/F ratios for severe ARDS in children based on "*The Berlin Definition*" is needed. A 2014 retrospective study on the relationship between ARDS according to Berlin criteria and mortality risk in children for 4 years concluded that using S/F ratio calculation to predict mortality resulted in a 2-fold higher mortality risk than using P/F ratio.[12]

The limitation of this study was that most PaO_2 values were above 100 mmHg. The PaO_2 values could exceed 100 mmHg, while SpO_2 values were limited to 100%. Hence, a comparison of these values would not yield a meaningful result. The mean PaO_2 value in this study was 100 mmHg, and only 3 subjects had PaO_2 values below 100 mmHg. According to the oxyhemoglobin curve, PaO_2 values >100 mm Hg cannot be used to determine a corresponding SpO_2 value. In conclusion, S/F ratio has a weak correlation with P/F ratio for ALI in children (r= 0.2).

Conflict of interest

None declared.

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Original Article

Differences in the stratum corneum of Indonesian infants and adults

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Abstract

Background Although understanding the stratum corneum (SC) of infant skin is important to avoid skin diseases such as atopic dermatitis, there has been no such investigation in Indonesian infants to date.

Objective To obtain a basic knowledge of SC characteristics in Indonesian infants in order to develop methods for infant-specific skin care and to prevent dermatitis and infection.

Methods Seventy-two healthy, full term infants aged 1 to 24 months who were native Indonesians residing in Jakarta were enrolled in this study. Some of the mothers were also enrolled in the study as adults (n=30). Transepidermal water loss (TEWL) and hydration of the SC (capacitance) on the thigh, buttock, and upper arm were measured after sufficient acclimation in an air-conditioned room, in both infants and mothers.

Results The SC hydration was significantly higher in infants than adults at all sites measured, including the buttocks, which is a diaper area. Infant TEWL values were also significantly higher than in adults at all sites. Hydration of the SC and TEWL values showed no significant correlation with age of infant for any site. The SC hydration and TEWL values of Indonesian infants did not decrease to adult values within 24 months, which indicates that the SC characteristics in infants continue to develop after 24 months of age.

Conclusion Indonesian infants aged 0-24 months have significantly higher SC hydration and TEWL values than Indonesian mothers. However, infant age has no correlation to SC hydration or to TEWL values. **[Paediatr Indones. 2017;57:35-40. doi: 10.14238/pi 57.1.2017.35-40]**.

Keywords: *infant skin, stratum corneum, Indonesian*

Hydration of the stratum corneum (SC) and transepidermal water loss (TEWL) are measurable parameters indicative of SC functions.[1,2] A decrease of the hydration value indicates the dryness of the SC, which occasionally leads to xerosis (dry skin) accompanied by desquamation, and/or pruritus. The TEWL value is a measurement of the barrier function of the SC. Elevated TEWL values indicate barrier disruption, which occasionally leads to an incursion of extraneous substances resulting in infections, contact dermatitis, and/or allergenic diseases.

Therefore, monitoring and understanding the SC properties of infant skin is important to avoid skin diseases, such as diaper dermatitis, infections, and atopic dermatitis. Indeed, many published studies have reported skin changes during infant growth and differences from adult skin, with regards to SC function and skin structure.[3-13] Many factors, such as age, body site, skin type, and ethnicity are likely to

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influence the changes in SC characteristics, namely, hydration expressed by capacitance (also known as the conductance value) and TEWL.

To date, there has no such study on SC characteristics in Indonesian infants. Hence, we investigated the SC changes during growth and the differences between Indonesian infant and adult skin in subjects residing in Jakarta. The purpose of this study was to obtain a basic knowledge of the SC traits in Indonesian infants in order to develop infant-specific skin care methods, skin-protective diaper formulations, as well as to prevent dermatitis and infection.

Methods

All clinical tests were performed according to the Declaration of Helsinki. All protocols were approved by the Ethical Committees of the Medical Faculty, University of Indonesia and Biological Science Research, Kao Corporation (Tochigi Japan). All protocols were submitted to the Indonesian national regulatory authority (*Badan Pengawas Obat dan Makanan*/BPOM) for approval. Subjects were recruited and their parents provided signed informed consent.

The source population of the study was eligible volunteers according to the inclusion criteria, who lived in Jakarta. This study was conducted at the clinical laboratory of Equilab International (Jakarta, Indonesia) in two separate, but similar, examinations, in November 2010 (1st examination: 30 infants and 30 their mothers as adults) and in March/April 2012 (2nd examination : 42 infants only). A total of 72 healthy, full term infants aged 1 to 24 months (35 girls and 37 boys) and 30 adults who were native Indonesians were enrolled in the study. Infants were biological children of the mothers, with proof of birth certificate. Infants who were genetically mixed with other races (non-Indonesian native races) such as Chinese, Arabic, American, etc. were excluded from the study. Subjects were from a middle-to-upper class socioeconomic status and used more than 2 diapers daily. Exclusion criteria were the presence of dermatitis at the sites of measurement, the use of systemic medicine within 2 weeks of the test, or those deemed inappropriate by the study physician.

All measurements were carried out in the same air-conditioned room, which was kept at 20±2°C and 50±10% relative humidity. All measurements were conducted in a similar manner using the same apparatus, technician, and conditions. After removing the infants' clothing, skin areas were wiped with a wet towel, left to dry for 20 minutes, then acclimated for 20 minutes. The measuring sites on the adults were treated in a similar manner. The TEWL was measured using a *Tewameter®* (Courage & Khazaka Electronic GmbH, Köln, Germany). Subsequently, the SC capacitance was measured using a *Corneometer®CM* (Courage & Khazaka), according to previously reported specifications.[14, 15]

Measurements were performed on subjects' skin of the inner thigh, buttock, and inner upper arm. Crying infants were not measured until they became quiet or fell asleep. Student's T-test or Tukey's test were used to determine statistical significance ($P<0.05$). Correlation coefficient values were determined by linear regression using the statistical function of Microsoft Excel. The statistical significance ($P<0.05$) of the correlation coefficient was determined by a correlation coefficient (r) table.

Results

The mean SC hydration (capacitance) and TEWL values of infants and adults are shown in **Figure 1**. The mean SC hydration in infants was significantly higher than in

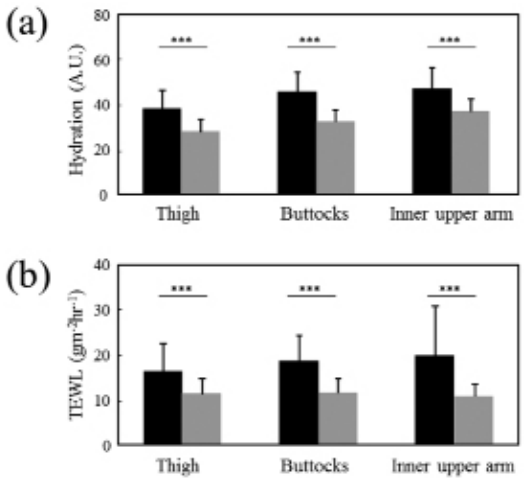

Figure 1. Stratum corneum characteristics of infants and adults (mother). (a) Hydration (Capacitance), (b) TEWL, solid bar ■: infants age in 1-24 months age (n=72), gray bar ■: adults (mother, n=30). Bars show means ± SD. Student-t test was used to determine statistical significance. ***p<0.001.

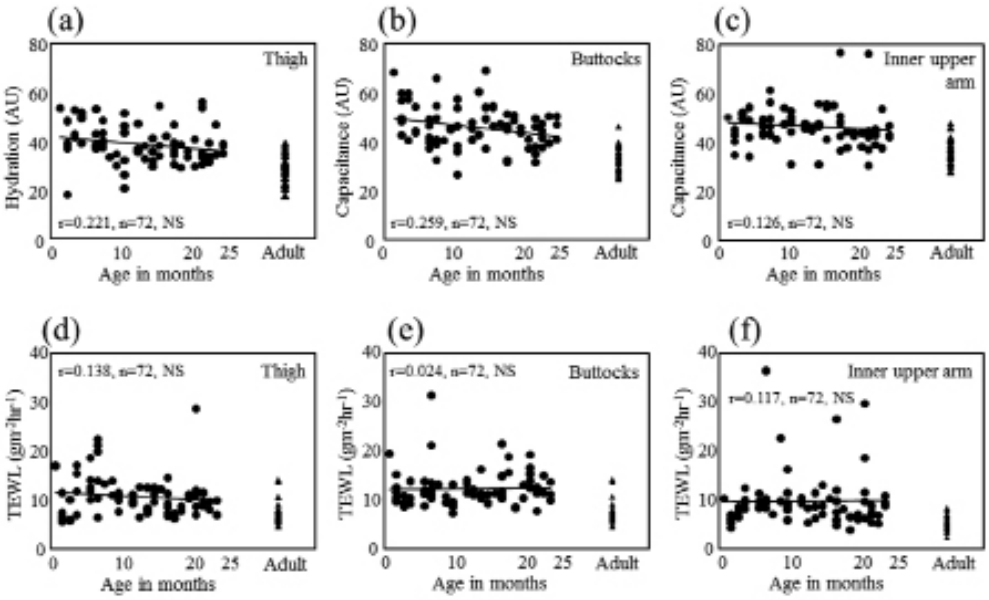

Figure 2. Individual plots of stratum corneum hydration and TEWL values versus age in month of infants on three sites.

(a), (b), (c): Hydration (Capacitance), (d), (e), (f): TEWL, (a)(d): thigh, (b)(e): buttocks, (c)(f): inner upper arm. Correlation coefficient values were determined by linear regression. The statistical significance ($p<0.05$) of the correlation coefficient was determined by a correlation coefficient (r) table. NS means not significant.

adults, at all sites, including the buttock (diaper area).

The mean TEWL value in infants was also significantly higher than in adults, at all sites.

Individual plots of SC hydration and TEWL values against age (in months) are shown in **Figure 2**. Hydration of the SC had no significant correlation with infant age at any of the sites. The TEWL value also had no significant negative correlations with infant age at any of the sites. The SC and TEWL values of the infants did not approach the adult values even at 24 months of age, which indicates that the growth of infant skin continues to develop after 24 months of age.

Discussion

In this study, we assessed the SC characteristics of 72 healthy, full term Indonesian infants aged 1 to 24 months. Hydration of the SC is a parameter that reflects skin dryness. Decreased SC hydration (capacitance) indicates a drier SC. It is well known that a dry environment and/or an exposure to a detergent often causes xerosis (dry skin) accompanied by desquamation and/or pruritus. Some types of dermatitis, such as atopic dermatitis, are commonly accompanied by the symptom of decreased SC hydration.

In infant skin, SC hydration tends to decrease within several days after birth, followed by an increase with post-neonatal age.[3-6,8,9] The SC hydration of infants aged 1 to 24 months has been reported as higher than[4,5,8,10] or similar to that of adults.[6] However, site-dependent differences have also been reported. A significant difference in capacitance between infant and adult skin was observed on the forearm skin, but not on the buttocks.[3] In addition, variations in changes with growth during the first 90 days of life on the forearm, buttocks, and facial skin have been reported.[4] Some differences in SC hydration between thigh skin and diaper-covered buttocks were also observed.[10]

In this study, Indonesian infants aged 1-24 months had significantly higher SC hydration than adults at all sites, including the buttocks (**Figure 1**). Infant age-related increases or decreases in capacitance were not noticeable (**Figure 2**). These results agree with most of previous reports, except for

some site-specific differences.[3,5,6,8,10] Collectively, it is likely that the SC in Indonesian infants is hydrated enough from even the 1st month of age and is stable to at least 24 months of age, the same as previously reported for other ethnicities. [5, 6, 11]

The TEWL value is generally used to evaluate the skin barrier function, especially for the inside-out barrier.[16] Elevated TEWL values indicate a disruption of the skin barrier, which occasionally leads to an incursion of extraneous substances resulting in infection, contact dermatitis, and/or allergenic diseases. The barrier function of the skin has an important relationship to the onset of allergic dermatitis in infants, which has been clarified clinically.[17,18]

Full-term neonates are reported to have sufficient barrier function of the skin.[19, 20] Recent studies have indicated that healthy neonates at the first 24 hours after birth have a much higher TEWL value than adults.[21, 22] Nikolovski *et al*. reported that infants (3 to 12 months of age) showed much higher TEWL values on their arm skin than adults.[5] Several ethnicities have higher TEWL values in infants than in adults, on the upper inner arm skin.[8] However, other reports have shown that infants have similar TEWL values to adults.[3,6] In general, no difference in TEWL values have been noted between the buttock and forearm skin,[3] although some differences between diaper-covered buttock skin and the thigh have been reported.[10]

In our study, Indonesian infants aged 1-24 months showed significantly higher TEWL values than the adults, at all sites including the buttocks. Infant age-related increases or decreases were not noticeable in TEWL values, similar to the SC hydration value results. However, some outliers were observed in the TEWL values at all sites (**Figure 2**). The TEWL value is a very sensitive parameter because it can be influenced by physiological and environmental factors, such as whether an infant is active or at rest, room temperature, and humidity. Therefore, these factors may be responsible for the inconsistency among past studies. Also, the differences in body site, age, measuring instruments, and living habits may have contributed to the inconsistency. Hence, TEWL results should be interpreted with care.

Hydration of the SC depends on two major factors: natural moisturizing factor (NMF, derived from filaggrin) and intercellular lipids (ICL). The amount of NMF changes dynamically with age, with newborns typically showing significantly higher levels of NMF than children of other ages and adults.[6] In contrast, another report showed that infants have significantly lower levels of NMF than adults at the SC surface.[5] Ceramide, a major constituent of ICL, is known to play key roles, not only in moisturizing, but also in skin barrier functions of the SC,[23, 24] but only a few studies have reported such. Minami-Hori *et al*. reported that ceramide content decreases remarkably with growth within the first 6 months after birth, and gradually becomes equal to adult values.[10] On the other hand, infants aged 6-24 months showed significantly lower levels of ceramide than children of other ages.[25]

A limitation of our study was the lack of clarifying the differences in SC characteristics of infants from those of adults specifically in terms of by two major factors: NMF and ceramide. Minami-Hori *et al*. reported that SC functions are not determined by single factors such as NMF, ceramides, etc., but are the result of the overall effects of numerous SC components that vary during growth.[10] In our study, NMF and ceramide levels in the SC were not determined. Further investigation is required to clarify the relationship between SC functions and biological factors, which apply to all ethnicities including Indonesian infants.

In conclusion, Indonesian infants aged 0-24 months have significantly higher SC hydration and TEWL values than Indonesian mothers. However, infant age has no correlation to SC hydration or to TEWL values We consider that Indonesian infants also need a lot of attention for skin treatments even after 24 months of age, the same as shown in other ethnicities.

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Conflict of interest

None declared.

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Original Article

Association between sleep quality and obesity in adolescents

Nova Juliana Sagala, Sri Sofyani, Supriatmo

Abstract

Background Sleep quality can be measured by the Pittsburgh Sleep Quality Index (PSQI). One component of the PSQI is duration of sleep, which is often highly inadequate in adolescents. Inadequate sleep may lead to obesity in adolescents.

Objective To assess for an association between sleep quality and incidence of obesity in adolescents.

Methods This case–control study was conducted at Santo Thomas I Senior High School, Medan, North Sumatera, from July to August 2015. A total of 227 adolescents were divided into two groups: the case group consisting of 101 obese adolescents and the control group consisting of 126 non-obese adolescents. Study data was collected by questionnaires and PSQI. We interviewed subjects on their food consumption for the three days prior and calculated their average caloric intake. The data were analyzed by non-paired T-test, Chi-square, Mann-Whitney, and multivariate analyses.

Results There was a significant association between sleep quality and obesity [OR 3.87 (95%CI 1.920 to 7.829)]. Median PSQI (range) score in the obese group was significantly higher than in the non-obese group [6.00 (2-16) vs. 5.00 (2-12), respectively (P=0.0001)]. In addition, sleep latency (P=0.002) and sleep duration (P=0.0001) were significantly different between groups. Multivariate analysis revealed a significant association between poor sleep quality and high caloric intake.

Conclusion Sleep duration in obese adolescents is significantly shorter than that in non-obese adolescents. In addition, sleep latency in obese adolescents was significantly longer than that in non-obese adolescents. **[Paediatr Indones. 2017;57:41-6. doi: 10.14238/pi 57.1.2017.41-6]**.

Keywords: *sleep quality; obesity; adolescents*

Sleep is a universal behavior in every animal species, and has been studied from insects to mammals. It is one of the most significant human behaviors, accounting for roughly one third of human life.[1] Sleep is generally considered to be a restorative process, having beneficial effects on immune function, and essential for physical growth, emotional stability, maintenance of cognitive function, and intellectual growth in adolescence.[2-4] The quality of sleep can be measured by the *Pittsburgh Sleep Quality Index* (PSQI).[5,6]

The most prominent changes in sleep patterns during adolescence are bedtime and waking time. Adolescents go to bed later and get up earlier because of school, and feel increasingly sleepy during the day.[7,8] Sleep deprivation has neurohormonal effects that can lead to increased caloric intake and subsequent obesity.[9,10]

This study was presented at the *Pertemuan Ilmiah Tahunan Ilmu Kesehatan Anak VIII/PIT IKA VIII* (The 8th Annual Scientific Meeting of Child Health), Makassar, September 19-21, 2016.

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Childhood obesity has reached epidemic levels globally. The *World Health Organization* (WHO) reported that an estimated 22 million children under 5 years of age and 10% of school-aged children between 5 and 17 years were overweight or obese.[9,11,12] Nutrition and physical activity have been the major focus of research on obesity prevention, while other risk factors such as sleep quality have received much less attention.[13]

We aimed to assess for an association between sleep quality and obesity in adolescents.

Methods

This case-control study was performed in Santo Thomas I High School, Medan, from July 2015 until August 2015. Subjects were students aged 15 to 18 years who provided informed consent. The exclusion criteria were adolescents with at least one obese parent, consumed corticosteroids, or had a family history of diabetes mellitus.

Subjects' weights and heights were measured and their body mass index (BMI) calculated and plotted on the *CDC 2000* BMI curve[14] Subjects were divided into two groups: the case group of obese adolescents and the control group of non-obese adolescents that included underweight, normal weight and overweight subjects. Subjects filled study data questionnaires and PSQI. Subjects' food consumption was verified by interview and we calculated the average caloric intake for the three days prior. A food model was used to help subjects describe the amount of food they had consumed.[15] *The Nutrisurvey Program* was used to calculate caloric intake. High caloric intake was defined to be a higher than average caloric intake, according to the Indonesian Ministry of Health Regulation [*Peraturan Menteri Kesehatan Indonesia (Permenkes)*] No. 13/1975. Non-high caloric intake was defined to be average or lower than average caloric intake, according to the Ministry of Health Regulation.[16]

The PSQI is an effective instrument for measuring quality of sleep. The PSQI can be used to differentiate "poor" and "good" sleep quality by measuring seven components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over a one-month period. A global score of ≤5 indicates a "good" quality of sleep, while global score of >5 indicates a "poor" quality of sleep, with higher scores reflecting poorer sleep quality.[6]

This study was approved by the Research Ethics Committee of the University of Sumatera Utara Medical School. Data were analyzed by non-paired T-test, Chi-square test, Mann-Whitney test, and multivariate analysis. Level of significance was defined by $P<0.05$ with 95% confidence intervals.

Results

A total of 227 adolescents who fulfilled the inclusion criteria were divided into two groups: the case group of 101 obese adolescents and the control group of 126 non-obese adolescents. Subjects' characteristics are described in **Table 1**. More males in obese group.

Differences in PSQI score and caloric intake between groups are shown in **Table 2**. Overall PSQI scores are a total of the individual component scores. Obese subjects had higher median sleep latency and duration scores than the non-obese subjects, indicating poorer quality in these two components of the PSQI. Median caloric intake was based on food consumption for three days and obese subjects also had significantly higher caloric intake than the non-obese group.

Table 1. Baseline characteristics of subjects

Characteristics	Obese group (n=101)	Non-obese group (n=126)
Gender, n (%)		
Male	62 (61.4)	50 (39.7)
Female	39 (38.6)	76 (60.3)
Median age (range), years	16.0 (15.0-17.9)	15.8 (15.0-17.9)
Mean body weight (SD), kg	87.5 (13.5)	60.5 (10.0)
Median body height (range), cm	165.0 (142-179)	162.0 (147-181)
Median BMI (range), kg/m^2	31.2 (24-47)	23.0 (17-29)

A comparison of sleep quality and obesity is shown in **Figure 1**. Poor sleep quality was found in 55.8% of obese subjects and 44.2% of non-obese subjects.

The associations between obesity and sleep quality as well as obesity and caloric intake are shown in **Table 3**. The risk of obesity in males with poor sleep quality was higher than that in females (OR 4.3 *vs.* 3.7, respectively). Caloric intake was categorized as

Table 2. The distribution of PSQI score and quantity of caloric intake between obese and non-obese group

Variables	Obese group (n=101)	Non-obese group (n=126)	P value
Median PSQI (range)	6.00 (2-16)	5.00 (2-12)	0.0001
Median PSQI components (range)			
Subjective sleep quality	1.00 (0-3)	1.00 (0-2)	0.348
Sleept latency	1.00 (0-3)	1.00 (0-3)	0.002
Sleep duration	2.00 (0-3)	1.00 (0-3)	0.0001
Habitual sleep eficiency	0.00 (0-3)	0.00 (0-2)	0.137
Sleep disturbance	1.00 (0-3)	1.00 (0-3)	0.291
Use of sleeping medication	0.00 (0-3)	0.00 (0-3)	0.088
Daytime dysfunction	1.00 (0-3)	1.00 (0-3)	0.464
Median caloric intake (range), kcal	2,682.40 (1,961-3,233)	2,212.20 (1,534-2,799)	0.0001

*Mann-Whitney test
0-3 value based on scoring PSQI questionnaire for every components
Subjective sleep quality : 0= very good 1= good, 2=poor, 3=very poor
Sleep latency : total score of
- Time falling asleep 0=<15 minutes 1=16-30 minutes 2=31-60 minutes 3= >60 minutes
- Cannot sleep in 30 minutes 0=not during the past month 1=less than once a week 2=once or twice a week 3=three or more times a week

Sleep duration 0=> 7 hours 1=>6-7 hours 2=5-6 hours 3=<5 hours
Sleep efficiency : 0: ≥85% 1=75-84% 2=65-74% 3=<65%
Sleep disturbance, Use of sleeping medication, Daytime dysfunction :
0=not during the past month 1=less than once a week 2=once or twice a week 3=three or more times a week

Table 3. The association of sleep quality and caloric intake with obesity

Variables	Obese group (n=101)	Non-obese group (n=126)	P value*	OR	95% CI
Sleep quality, n(%)					
Male					
Poor	45 (72.6)	19 (38.0)	0.001	4.3	1.94 to 9.60
Good	17 (27.4)	31 (62.0)			
Female					
Poor	32 (82.1)	42 (55.3)	0.005	3.7	1.45 to 9.42
Good	7 (17.9)	34 (44.7)			
Caloric intake, n(%)					
High	91 (90.1)	61 (48.4)	0.0001	9.7	4.62 to 20.33
Not high	10 (9.9)	65 (51.6)			

*Chi-square

Table 4. The association between sleep quality and caloric intake

Sleep quality	High caloric intake (n=152)	Not high caloric intake (n=75)	P value*	OR	95% CI
Poor	103 (67.8)	35 (46.7)	0.002	2.40	1.36 to 4.23
Good	49 (32.2)	40 (53.3)			

*Chi-square

high or not-high. A significantly higher percentage of obese subjects (both males and females) had poor sleep quality than did non-obese subjects [males: 72.6% *vs.* 38.0%, respectively (P=0.001); females: 82.1% *vs.* 55.3%, respectively (P=0.005)].

The association between sleep quality and caloric intake is shown in **Table 4.** There was a significant association between poor sleep quality and high caloric intake (OR 2.40; P=0.002).

Multivariate analysis of variables that contributed to obesity with P<0.25 is shown in **Table 5**. Overall, high caloric intake was the strongest factor that contributed to obesity.

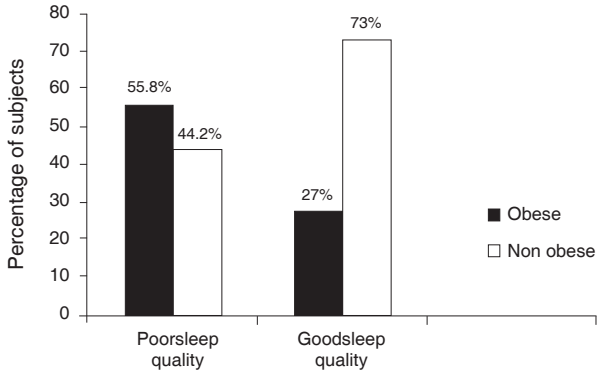

Figure 1. Comparison of obesity and sleep quality

Discussion

Obesity is the accumulation of excess body fat which may adversely affect health.[17] High rates of childhood obesity have been evident not only in developed countries, but in recent years developing countries have also joined the trend.[18] The prevalence of obesity in Indonesia has reached 10.3%, with nearly the same incidence reported in Medan (10.4%).[19,20] In this study, the mean body weight of both the obese and non-obese groups exceeded normal body weight, according to *Angka Kecukupan Gizi* (recommended daily allowance/ RDA) recommendations, that is, 46-56 kg for males and 46-50 kg for females.[16] The median body heights of both groups were appropriate, for age according to RDA recommendations, that is, 155-165 cm. The BMI in both groups exceeded the RDA recommendation, that is, 18.42-20.56 kg/ m2 for males and 19.14-20.02 kg/m2 for females.[16] This result may have been due to the inclusion of overweight subjects in the non-obese group.

We found that the risk of obesity in males was greater than that in females. Our finding is consistent with studies in Brazil and South Korea, which found that 53% of obese adolescents were male. The higher likelihood of being overweight or obese among male youths may be associated with testosterone, which is actively produced during puberty. Testosterone decreases serum leptin secretion by 62%.[21,22] Leptin is a hormone that controls satiety. Low leptin levels lead to an increase in appetite.[11,21] Several epidemiological studies have noted links between obesity and sleep.[12]

Table 5. Multivariate analysis of factors that contribute to obesit

Variables	P value	OR	95% CI
Sleep quality	0.0001	3.87	1.92 to 7.83
Gender	0,0001	4.02	2.03 to 7.97
Caloric intake	0.0001	12.03	5.23 to 27.66

*Chi-square

The major sleep problem among adolescents is sleep deprivation. 23 Sleep duration also contributes to sleep quality.[6,12]

In our study, most adolescents had poor sleep quality, and the median PSQI score in the obese group was significantly higher than that in the non-obese group. A study in Lebanon found that 58.7% of adolescents had poor sleep quality with the mean PSQI score was 6.57 and a range of PSQI scores of 3 to 10. 24 In addition, a Texas study stated that in obese woman aged 16 - 40 years, the mean PSQI score was 6.2. 25 Our median PSQI scores were similar to that in the Lebanon (6.57) and Texas studies.

Shorter sleep duration is associated with increased ghrelin levels and decreased leptin levels. Ghrelin increases the appetite, whereas leptin reduces it; hence, these hormonal changes lead to an increase in appetite.[25-27] Furthermore, later bedtimes may provide more opportunities to eat. These conditions together tend to lead to an increased caloric intake. Short sleep duration also causes tiredness and daytime drowsiness, which in turn can lead to decreased physical activity and a preference for watching television.[27] Finally, some evidence has shown that when people sleep less, their core body temperature drops. All of these mechanisms (increased calori intake and decreased physical activity) have roles in altered energy expenditure.[9]

We found a significant difference in sleep duration between the two groups. The median score of sleep duration in obese group was 2 which means that the obese group had a sleep duration of 5 to 6 hours, whereas median score in the non-obese group was 2 which means the non obese had sleep duration ≥6 to 7 hours. Similarly, previous studies showed that increased BMI was associated with shorter sleep duration.[28,29]

Sleep latency was also significantly different between the two groups. A Sao Paulo study in obese adolescents aged 15 to 19 years reported that 63.3%

of subjects slept less than 8 hours per day, and 87.27% of the subjects reported that their sleep latency was more than 30 minutes.[30] We found that 55.8% of subjects with poor sleep were obese. Previous studies in Pekanbaru and Atlanta, reported that 39.4% and 43.7% of poor sleepers were obese.[31,32]

Several limitations in this study were the case-control method, the dependence on subjects' memory for data recollection, which may have been subject to recall bias, and the lack of data on daily physical activity and sedentary lifestyle.

In conclusion, there is a significant relationship between poor sleep quality and obesity in adolescents. Sleep duration in obese adolescents is significantly shorter than that in non-obese adolescents. In addition, sleep latency in obese adolescents was significantly longer than that in non-obese adolescents.

Conflict of Interest

None declared.

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Original Article

A comparison of axillary and tympanic membrane to rectal temperatures in children

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Abstract

Background *Core body temperature measurement is not commonly done in pediatric populations because it is invasive and difficult to perform. Therefore, axillary and tympanic membrane temperature measurements are preferable, but their accuracy is still debatable.*

Objective *To compare the accuracy of axillary and tympanic temperatures to rectal temperature in children with fever, and to measure the cut-off point for fever based on each temperature measurement method.*

Methods *A diagnostic study was conducted among feverish children aged 6 months to 5 years who were consecutively selected from the Pediatric Outpatient Clinic, Pediatric Emergency Unit, and the inpatient ward in the Department of Child Health, Cipto Mangunkusumo Hospital (CMH), from December 2014 to January 2015. Subjects underwent three measurements within a two minute span, namely, the axillary, tympanic membrane, and rectal temperature measurements. The values obtained from the examination were analyzed with appropriate statistical tests.*

Results *The cut-off for fever on axilla was 37.4oC and on tympanic membrane was 37.4oC, with sensitivity 96% (95%CI 0.88 to 0.98) and 93% (95%CI 0.84 to 0.97), respectively; specificity 50% (95%CI 0.47 to 0.84) and 50% (95%CI 0.31 to 0.69), respectively; positive predictive value/PPV 90% (95%CI 0.81 to 0.95) and 85% (95%CI 0.75 to 0.91), respectively; and negative predictive value/NPV 83% (95%CI 0.61 to 0.94) and 69% (95%CI 0.44 to 0.86), respectively. The optimal cut-off of tympanic membrane and axilla temperature was 37.8oC (AUC 0.903 and 0.903, respectively).*

Conclusion *Axillary temperature measurement is as good as tympanic membrane temperature measurement and can be used in daily clinical practice or at home. By increasing the optimum fever cut-off point for axillary and tympanic membrane temperature to 37.8oC, we find sensitivity 81% and 88%, specificity 86% and 73%, PPV 95% and 91%, and NPV 95% and 91%, respectively.* **[Paediatr Indones. 2017;57:47-. doi: 10.14238/pi 57.1.2017.47-]**.

Keywords: *children; axillary temperature; tympanic temperature; rectal temperature; fever*

Fever is defined as a rectal temperature ≥ 38°C, axillary temperature ≥ 37.4°C, and tympanic membrane temperature ≥ 37.6°C.[1,2] Previous studies reported that axillary temperature is 0.72 to 0.85°C lower than rectal temperature, and 0.55°C lower than tympanic membrane temperature; in addition, tympanic membrane temperature is 0.17 – 0.49°C lower than rectal temperature.[3,4] Another study reported 0.2 – 0.6°C differences between axillary and tympanic membrane temperatures.[5]

Currently, there are no data nor studies comparing the accuracy of tympanic membrane and axillary temperature to rectal temperature as the gold standard of core body temperature in children and adults. Therefore, we aimed to determine the accuracy of axillary and tympanic membrane temperatures compared to rectal temperature, in feverish children aged 6 months to 5 years who visited the Pediatric Outpatient Clinic, Pediatric Emergency Department,

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and the inpatient Pediatrics Ward at Dr. Cipto Mangunkusumo Hospital, Jakarta.

Methods

This diagnostic study was performed to determine the accuracy of axillary and tympanic membrane temperature results compared to rectal temperature in feverish children aged 6 months to 5 years, who visited the Pediatrics Outpatient Clinic, Pediatrics Emergency Unit, and the inpatient Pediatrics Ward in the Department of Child Health, Dr. Cipto Mangunkusumo Hospital, from December 2014 to January 2015. The temperature of the tympanic membrane was measured using infrared thermometers (OMRON Gentle Temp 510), while the axillary and rectal temperatures were measured with digital thermometers (*OMRON MC 246*). The temperature measurement was done by trained doctors. The minimum required sample size was 90 subjects, who were consecutively selected. This study was approved by the Research Ethics Committee of the University of Indonesia Medical School, and subjects' parents provided informed consent.

Room temperature was measured with a GEA thermometer 10 minutes before the patient's body temperature measurement, and insulation was put around the subject's bed. Thermometers were calibrated and cleaned beforehand. Disposable probe covers for the infrared thermometer were used for each patient. Before taking measurements, the six steps of hand washing were performed followed by wearing clean latex gloves. Subjects underwent three measurements within a two minute span, namely, the axillary, tympanic membrane, and rectal temperature measurements. The values obtained from the examination were analyzed with appropriate statistical tests.

Results

During the study period, of 280 patients who came to the Pediatric Outpatient Clinic with complaints of fever, 78 were aged over 5 years, 91 were in a state of neutropenia or thrombocytopenia, 18 experienced emergency situations (shock, tachypnoea, or tightness), and 3 had anorectal anatomical abnormalities or were surgerical. Hence, a total of 90 infants and children were enrolled. Subjects underwent axillary, tympanic membrane, and rectal body temperatures measurements and consisted of 42% males and 58%females. They comprised the following age ranges: 6 months - 1 year (19%), >1 year - 3 years (38%), and >3 years - 5 years (43%). Initial fever detection was done by measuring body temperature using a thermometer (67%) and palpation (29%), prior to the study. Types of thermometer used by parents were digital (62%) and mercury (6%). Treatments given to manage fever were antipyretics (42%), warm water compresses (6%), and nothing (2%). Antipyretics used was paracetamol (100%) syrup (87%). Ninety-six percent of parents were aware of the dangers due to fever.

Diagnostic results comparing axillary (37.4°C) and rectal temperature measurements had a sensitivity of 96% (95%CI 0.88 to 0.98), specificity of 50%

Table 4. Summary of diagnostic results of axillary and tympanic membrane temperatures at various cut-off points compared to rectal temperatures

Diagnostic results \ Cut off temperature	37.4°C	37.5°C	37.6°C	37.7°C	37.8°C
Axillary temperature					
Sensitivity, %	96	93	90	88	81
Specificity, %	50	68	73	77	86
PPV, %	90	90	91	92	95
NPV, %	83	75	70	68	60
Tympanic membrane temperature					
Sensitivity, %	95	94	93	91	88
Specificity, %	36	50	50	60	73
PPV, %	84	85	85	87	91
NPV, %	56	73	69	68	66

(95%CI 0.47 to 0.84), PPV of 90% (95%CI from 0.81 to 0.95), NPV of 83% (95%CI 0.61 to 0.94), positive likelihood ratio (PLR) 3 (95%CI 1.52 to 5.92), and negative likelihood ratio (NLR) 0.06 (95%CI 0.02 to 0.2). Diagnostic results of tympanic membrane temperature (37.6°C) compared to rectal measurements were sensitivity of 93% (95%CI 0.84 to 0.97), specificity of 50% (95%CI 0.31 to 0.69), PPV of 85% (95%CI 0.75-0.91), NPV of 69% (95%CI 0.44 to 0.86), PLR 1.85 (95%CI 1.13 to 3.04), and NLR 0.15 (95%CI 0.06 to 0.38) (**Table 1**).

The ROC curve of axillary and tympanic membrane temperature showed good AUC value of 0.903 for axillary temperature and 0.885 for tympanic membrane temperature.

Discussion

In this study, subjects' fever was first detected by parents' temperature measurement with thermometers (67%) or by palpation (29%). Of the 67% of parents who had thermometers at home, 92% had digital, 8% had mercury, and none had infrared thermometers at home. In developing countries, including Indonesia, not all families have thermometers, so fever assessment may be reliant on the perception of mother/caregiver by palpation. Thermometer prices play a role in the availability of thermometers at home. Infrared thermometer prices range from Rp 400,000 to Rp 1,500,000 with the probe cover price of Rp 350,000 / 20 probe covers. Hence, infrared thermometers are about 8-15 times the price of digital thermometers, and 40-150 times the price of mercury thermometer. Studies have found that although the temperature assessment by palpation is good enough to detect fever (sensitivity 89.2 to 96.3% and specificity 23 to 64.3%), the assessment is influenced by subjectivity, technique, and environmental factors.[8-11] Banco *et al.* reported that detection of fever by mothers without using a thermometer had a sensitivity of 73.9% and a specificity of 85.6%.[12]

As many as 90% of children aged ≤ 2 years with temperature ≥ 38.9°C were diagnosed as having fever, and 52.3% of children who complained of suffering from a fever were actually proven to have fever when the temperature measurement was taken with thermometer. A previous study found that 82% of 264 caregivers of children taken to the ER were very worried by the presence of fever, with one-third of the caregivers deciding that the child needed to be treated even if the measured temperature was less than 37.9°C.[13-16] In our study, 96% of parents were aware of the dangers of fever and gave antipyretic drugs if the body temperature was >37.5°C (90%). Also, one-third (31%) of parents gave antipyretic drugs a few hours before the child was brought for treatment (mean 3.7 hours). The prevailing parental perception was that the child's body temperature should be within the normal range (36.5 – 37.5°C). Many parents and caregivers had a phobia of fever, although fever is actually the body's physiological mechanism against infection. Thus, counseling and education for parents are needed to explain that the primary purpose of fever management was not only to lower the body temperature to normal limits, but also make the child more comfortable and ensure adequate intake of fluids and nutrients. Parents also need to be educated on the proper storage of antipyretic drugs.

The accuracy of axillary and tympanic membrane temperature measurement results remain inconclusive. In our study, mean axillary temperature was 0.5°C lower than mean rectal temperature and 0.3°C lower than mean tympanic membrane temperature. Mean tympanic membrane temperature was 0.2°C lower than the mean rectal temperature. Similarly, previous studies reported that axillary temperature was 0.25 - 1°C lower than rectal; axillary temperature was 0.18 – 0.55°C lower than tympanic membrane temperature; and the tympanic membrane temperature was 0.17-0.49°C lower than rectal temperature.[2-4,17-23]

A systematic review by Craig et al. reported a 0.25°C difference between axillary temperature - measured with a rectal mercury thermometer and 0.85°C with digital thermometer. Mean differences of axillary-rectal temperatures were 0.17°C for neonates and 0.92°C for children and adolescents.[24] Variations in temperature can be caused by different types and brands of thermometers, malposition of the digital thermometer tip at the time of measurement, and improper ear tug technique.

Diagnostic values obtained with the fever cut-off of 37.4°C (axilla) and 37.6°C (tympanic membrane) were: sensitivity of 96% (95%CI 0.88 to 0.98) and 93% (95%CI 0.84 to 0.97), specificity of 50% (95%CI 0.47 to 0.84) and 50% (95%CI 0.31 to 0.69), PPV of

90% (95%CI 0.81 to 0.95) and 85% (95%CI 0.75 to 0.91), and NPV of 83% (95%CI 0.61 to 0.94) and 69% (95%CI 0.44 to 0.86). In our study, if the fever cut-off of axillary and tympanic membrane temperature was raised to 37.8oC or 37.7C (**Table 1**) diagnostic values obtained were: sensitivity of 81% (95% CI 0.7-0.8) and 88% (95%CI 0.78 to 0.94), specificity of 86% (95%CI 0.67 to 0.95) and 73% (95%CI 0.52 to 0.87), PPV of 95% (95%CI 0.86 to 0.98) and 91% (0.82 to 0.96), and NPV of 60% (95%CI 0.42 to 0.74) and 67% (95%CI 0.47 to 0.82).

A previous study reported that the results of axillary temperature measurements were as good as tympanic membrane temperature results, with a sensitivity 94% and 70%, respectively, and specificity of 92% and 94%, respectively.[25] Other studies reported that the temperature of the tympanic membrane had a 67-76% sensitivity for detecting fever in children aged 6 months - 6 years and there was no significant difference between the results of temperature measurements between the right and left ear (0.019 – 0.2°C).[3,28-30]

To minimize measurement errors, axillary skin must be dry. The precision of axillary temperature measurement for detecting fever is affected by peripheral vasoconstriction at the initial onset of fever, sweating, and evaporation resulting in lower skin temperature compared to the actual body temperature. Tympanic membrane temperature accuracy is affected by the technique of ear tugging and probe position so the infrared thermometer probe needs to be appropriately-sized for the the diameter of the ear canal. Differences in brand, variation, and type of thermometer used contributed to the variations in measurement results generated from this study compared to previous studies, therefore, to avoid bias, temperature measurements in this study were conducted by a research assistant (physician) who had previously been trained and undergone validation tests. In addition, the thermometer battery was replaced every day and disposable probe covers were replaced for each subject.

In conclusion, the optimum cut-off point for diagnosing the presence of fever measured by axillary and tympanic membrane temperatures was 37.8°C, with fair sensitivity, specificity, and PPV (axillary: 81%, 86%, and 95%, respectively, and tympanic membrane of 88%, 73%, and 91%, respectively). Axillary temperature measurements were equally comparable to that of the tympanic membrane for detecting fever and can be used in everyday clinical practice or at home.

We suggest that clinicians use a fever cut-off point of ≥ 37.8°C for clinicians and ≥ 37.5°C for parents. Pediatricians should be able to explain the definition of fever and the appropriate time to give antipyretic drugs to parents.

Conflict of Interest

None declared.

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Original Article

Puberty onset in rural and urban children

Hariadi Edi Saputra, Hakimi, Melda Deliana, Siska Mayasari Lubis, Karina Sugih Arto

Abstract

Background Accelerated pubertal onset has been reported in recent years. Environmental factors are assumed to influence this condition.

Objective To assess differences in pubertal onset between children in rural and urban areas, as well as to evaluate body mass index (BMI) and socioeconomic status that affect pubertal onset.

Methods This cross-sectional study was conducted in July 2010 at junior high schools in Mandailing Natal and Medan, North Sumatera. Data were collected with purposive sampling of children aged 8 to 13 years. Sexual maturity was assessed by Tanner stage and risk factors was determined by questionnaires. The comparison between pubertal onset in rural and urban areas was assessed by Mann-Whitney U test. The relationships between nutritional status, socioeconomic status, sexual maturity, and pubertal onset were assessed by Spearman's correlation.

Results Eighty-four subjects (38 boys and 46 girls) from a rural area and 87 subjects (40 boys and 47 girls) from an urban area participated in this study. There were significant differences in mean pubertal age of onset between subjects living in rural vs. urban areas, for both girls and boys [girls: 10.2 vs. 9.5 years, respectively (P=0.008); boys: 11.7 vs. 10.1 years, respectively, (P=0.001)]. We found weak negative correlations between BMI and pubertal onset in boys (r=-0.246; P=0.03) and in girls (r=-0.548; P=0.001). We also found weak negative correlations between socioeconomic status and pubertal onset in boys (r=-0.406; P=0.0001) and in girls (r=-0.575; P=0.001).

Conclusion Pubertal onset is faster in girls and boys who lived in an urban area. There are negative correlations between BMI and socioeconomic status with pubertal onset. **[Paediatr Indones. 2017;57:52-6. doi: 10.14238/pi 57.1.2017.52-6]**.

Keywords: *pubertal onset; children; urban; rural*

In some countries, there has been an acceleration of puberty in boys and girls in recent decades.[1,2] This situation occours due to the possibility of increasing socioeconomic conditions, nutrition, psychologic stimulation, health, urban and rural areas.[2-4] Changes in attitude and behavior towards a more advanced and healthy lifestyle, as well as diet and nutrition, have impacted the health of certain groups. Obesity has impact on child develop, including onset of puberty.[4]

Incidence of pubertal disorders differs between sexes. The incidence of precocious puberty was ten times greater in girls than in boys.[5] Pubertal age is also influenced by ethnicity, and this may be caused by differences in BMI between races. Onset of puberty was associated with greater BMI. Onset of puberty in girls starts with the development Tanner stage breast 2. Normal puberty in girls begins at the age of 8 to 13 years. Onset puberty in boys, if testical volume more than 3 mL and age of onset puberty in boys is 10 to 14 years.[1] Pubertal acceleration will cause

This study was presented at the *Kongres Nasional Ilmu Kesehatan Anak/ KONIKA XV* (The 15th Child Health National Congress), Manado, July 11–14, 2011.

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pubertal hormonal changes, both qualitatively and quantitatively, resulting in rapid weight and height gain.[5] The aim of this study was to assess differences in pubertal onset between children who lived in rural and urban areas. We also evaluated BMI and socioeconomic status that affected pubertal onset.

Methods

We conducted a cross-sectional study from May to June 2010, in elementary schools at Gunung Baringin (rural area) and Medan (urban area), North Sumatera Province. Inclusion criteria were girls and boys 8 to 13 years with Tanner sexual scale of stage 2 or more. Exclusion criteria were the long term use of steroids, precocious or delayed puberty, use of chemotherapy or radiotherapy, use of hormonal drugs, chronic diseases, dismorphic diseases, orchitis, kryptorchismus and phymosis.

Height was measured by 2M microtoise (sensitivity 0.5 cm) and weight was measured by a pair of *Camry®* scales (sensitivity 0.1 kg). Body mass index (BMI) was calculated and plotted on the *CDC 2000* BMI growth charts.[6] Sexual maturity in both girls and boys was determined by Tanner scale assessment.[7] We also assessed testicular volume with Prader orchidometry and measured penile length with a wooden spatula. This study was approved by the Health Research Ethics Committee at the University of Sumatera Utara Medical School.

We used Mann-Whitney U test to assess the difference between onset of puberty in urban and rural areas. Spearman's correlation test was used to assess relationships between BMI, socioeconomic status, and onset of puberty. The limit of significance was $P<0.05$.

Results

Of 85 subjects in rural children, 1 boy was excluded because of orchitis and 87 subjects in urban children, 2 boys were excluded because of kryptorchismus (**Figure 1**). In this study, the approximate minimal sample were 36 boys and 43 girls in overall area that fulfill the criteria. The general characteristics of subject study between rural and urban children are shown in **Table 1**.

Tanner stages in rural and urban areas children are shown in **Table 2.** There was a difference in physical and sexual characteristics between boys ($P=0.001$) and no difference in girls ($P=0.112$) (**Table 2**).

Relationship between BMI, socioeconomic status and puberty in children are shown in **Table** 3. We found in girls a significantly negative correlation ($P<0.001$ and $r = -0.548$) between age at onset of

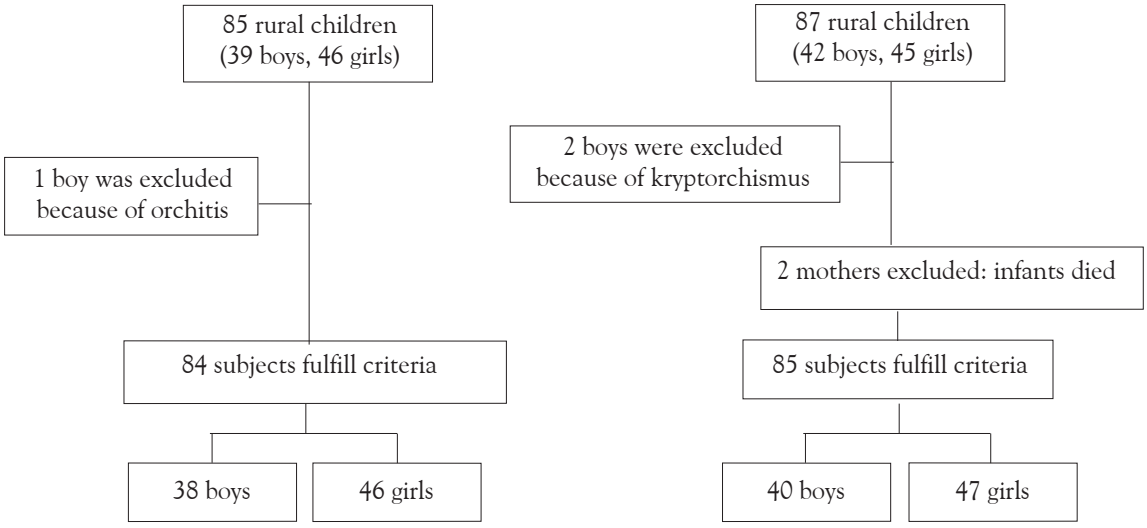

Figure 1. Study profile

Table 1. Characteristics of subjects

Characteristics	Boys		Girls	
	Rural (n=38)	Urban (n=40)	Rural (n=46)	Urban (n=47)
Mean age (SD), years	11.3 (1.45)	9.9 (0.61)	10.2 (1.22)	8.4 (3.24)
Mean body weight (SD), kg	24.1 (4.98)	27.5 (9.45)	25.3 (6.26)	24.7 (5.52)
Mean height (SD), m	1.2 (0.12)	1.2 (0.180	1.3 (0.11)	1.3 (0.15)
Mean body mass index (SD), kg/m^2	14.2 (2.21	15.8 (3.42)	14.6 (2.44)	15.1 (2.92)
Nutritional status, n				
Underweight	20 (25.6)	10 (12.8)	18 (19.8)	15 (16.3)
Normoweight	18 (23.1)	27 (34.6)	27 (30.2)	27 (29.1)
Overweight	0	3 (3.8)	1	5 (4.7)
Mean monthly income (SD), IDR	144,177 (139.42)	276,608 (177.43)	195,940 (157.94)	211,110 (120.71)

Table 2. Physical and sexual development of subjects

Tanner stage, n(%)	Boys		P value	Tanner stage, n(%)	Girls		P value
	Rural (n=38)	Urban (n=40)			Rural (n=46)	Urban (n=47)	
G2P1	34 (43.6)	21 (26.9)	0.001	M2P1	33 (35.4)	36 (38.7)	0.112
G2P2	3 (3.8)	17 (21.8)		M2P2	10 (10.8)	5 (5.4)	
G3P2	1 (1.3)	1 (2.6)		M3P1	0	4 (4.3)	
				M3P2	3 (3.2)	2 (2.2)	

Table 3. The relationships between pubertal onset and nutritional and socioeconomic status

Variables	Boys	P value	Girls	P value
	Coefficient correlation (r)		Coefficient correlation (r)	
Body mass index	-0.246	0.03	-0.548	0.001
Economic status	-0.406	0.001	-0.575	0.001

Table 4. Differences in pubertal onset between boys and girls who lived in rural and urban areas

Variables	Boys		P value	Girls		P value
	Rural (n=38)	Urban (n=40)		Rural (n=46)	Urban (n=47)	
Median pubertal onset (range), years	11.7 (9.0-13.6)	10.1 (9.0-11.2)	0.001	10.2 (8/0-12/6)	9/5 (8/0-12)	0.008

puberty with BMI, the higher BMI associated with earlier puberty. We also found in boys and girls a significantly negative correlation between age onset of puberty with economic status, the higher income associated with earlier puberty.

Differences in pubertal onset between boys and girls who lived in rural and urban areas are shown in **Table 4**. There were significant differences in the mean age of pubertal onset. Pubertal onset in boys and girls from urban area were faster than those from rural area.

Discussion

We determined rural and urban areas based on a scoring system which was developed by the National Statistical Agency in 2000.8 Scores were based on population density and the percentage of households that have telephones, electricity, and the supporting urban facilities.[8] Gunung Baringin Village, Panyabungan East District, Mandailing Natal Regency, classified as rural because it had a score of less than 10, while the Maimoon District, Medan

municipality had a score over 10. The poor are people who have an average income below the poverty line (Rp. 262,262 per capita per month in 2008 - 2009).[9] In this study, we observed low incomes below the poverty line in both rural and urban areas, [boys in rural areas: Rp. 144,177 (SD 139.4); girls in rural areas: Rp. 195,940 (SD 157.9); girls in urban areas: Rp. 211,110 (SD 120.7)].

One of the impact of obesity was earlier pubertal onset.[10] BMI represents body fat, which is shown in two studies of 100 boys and 92 girls between the ages of 7 to 17 years. The correlation between BMI and fat mass (measured using dual-energy radiograph absorptiometry) in girls was 0.94 in White girls and 0.96 in Black girls. The Correlation of BMI and fat mass was 0.83. The correlation between BMI and body mass in boys was 0.85, while the correlation of BMI to body fat is 0.54.[11,12] Our study showed mean BMI values were significantly different between the boys in urban and rural areas, However, this difference was not observed in girls.

In 1997, an *American Academy of Pediatrics Pediatric Research in Office Settings* (PROS) study of 17,000 girls in the United States found that the mean age of onset of puberty was 10 years in white American girls and 8.9 years in African-American girls.[13] A 1970 study in boys in England observed that the mean age of onset of puberty was 11.6 years.[14] Similarly, the mean age of pubertal onset was 11.5 years in the US in 1985,[15] 11.6 years in Sweden in 1996,[16] and 11.5 years in the Netherlands in 2001.[17] A 2005 study in Indonesia found the mean age of pubertal onset to be 11-12 years.[18] A study in West Sumatera found a mean age of pubertal onset in boys from urban areas was lower than suburban areas [112.26 (SD 21.77 months *vs.* 119 (SD 19.65) months]. The mean age of pubertal onset in girls from urban areas is also lower than suburban areas [113.56 (SD 21.9) months *vs.* 115.6 (SD 18.78) months].4 This situation occurs due to differences in socioeconomic and nutrition conditions. Previous studies have found that children in cities experienced earlier puberty than children in villages.[19,20] Our study also showed faster pubertal onset boys and girls who lived in urban area.

Children with good nutritional status may experience earlier puberty than children with less nutrition.[21-23] A hypothesis said that obesity can trigger the neuroendocrine system to start puberty.[24] Age of puberty was also influenced by ethnicity, and this may be due to differences in BMI between the races. Several studies also found associations between onset of puberty and BMI.[1,4,12] Some studies also showed correlations between adolescent BMI and pubertal onset.[25,26] A 2008 study reported that a one-unit increase in age between 2 and 8 years was associated with sooner growth spurt at puberty, approximately ±0.6 years in boys and ±0.7 years in girls.[11] A 2009 study in Semarang in 502 children found a significantly negative correlation ($r=-0.49$; $P<0.001$) between age at onset of puberty with BMI, The higher BMI associated with earlier puberty.[26] Our study also found relationship between BMI and age of pubertal onset, especially in girls with greater BMI caused early onset of puberty.

A study in Semarang found a strong correlation between socioeconomic status and the onset of puberty ($r=-0.64$; $P<0.001$).[26] Study in Kosovo found that socioeconomic factors influenced differences in the quality and quantity of food intake. Girls with less food intake experienced menarche in 13.[29] years, while girls with good food intake experienced menarche in 12.91 years.[27] A California study found girls from high family incomes (over $75,000) had earlier onset of puberty than girls from low income families (less than $75,000).[12] Problems related to puberty were physical appearance, pregnancy, sexually transmitted diseases, sexual abuse, drug abuse, eating disorders, depression, and obesity.[28] Earlier onset of puberty was also associated with increased risk of psychological disorders, such as post-traumatic stress disorder (PTSD), specific phobias, and social anxiety disorder (SAD).[29] We found a moderate relationship between age at pubertal onset in boys and socioeconomic level. We also found negative correlations between socioeconomic status and pubertal onset in boys ($r=-0.406$; $P=0.0001$) and in girls ($r=-0.575$; $P=0.001$).

In conclusion, pubertal onset are faster in girls and boys who lived in an urban area compared to those in rural area. We also find negative correlations between nutritional status and socioeconomic status with pubertal onset.

Conflict of Interest

None declared.

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Original Article

Glycated hemoglobin HbA1c, waist circumference, and waist-to-height ratio in overweight and obese adolescents

Elysa Nur Safrida, Neti Nurani, Madarina Julia

Abstract

Background Central obesity has been associated with a high risk of insulin resistance. Waist circumference and waist-to-height ratio are anthropometric indices for determining central obesity and have been associated with increased blood pressure, cholesterol, and insulin levels. In adults, fat distribution around the waist is a valid predictor of glycated hemoglobin (HbA1c)levels, and is currently recommended by experts as a diagnostic tool for diabetes. Central obesity measurement has advantages over fasting blood glucose and oral glucose tolerance tests, as it is simple and inexpensive to perform.

Objective To assess for correlations between HbA1c level and waist circumference as well as waist-to-height ratio and to assess factors potentially associated with HbA1c levels in overweight and obese adolescents.

Methods This cross-sectional study was done in four junior high schools in Yogyakarta, which were obtained by cluster sampling. Overweight and obese students who were generally healthy were included in the study. Subjects underwent waist circumference and waist-to-height ratio measurements, as well as blood tests for HbA1clevels.

Results Sixty-seven children participated in the study, with 48 girls (71.6%) and 19 boys (28.4%). Waist circumference and HbA1c levels were not significantly associated (r=0.178; P=0.15). However, waist-to-height ratio and HbA1c levels had a weak positive correlation (r=0.21; P=0.04). Linear regression analysis revealed that waist-to-height ratio had a significant association with HbA1c level (P=0.02), but age, sex, and nutritional status did not.

Conclusion Waist-to-height ratio is correlated with HbA1c levels in overweight and obese adolescents. **[Paediatr Indones. 2017;57:57-62. doi: http://dx.doi.org/10.14238/pi57.2.2017.57-62]**.

Keywords: *waist circumference; waist-to-height ratio; glycated hemoglobin; obesity; adolescent*

The number of obese and overweight children has increased more than 100% in the past 30 years.[1] According to the *World Health Organization* (WHO) in 2010, more than 40 million children under 5 years of age were overweight.[2] In Indonesia, about 14% of children and 19.1% of adolescents were categorized as overweight, while the national prevalence of obesity in children aged 13-15 years reached 2.5%.[3] A Yogyakarta study in junior high school students reported that 6.41%of adolescents were obese.[4]

Obesity in childhood often continues into adolescence and adulthood.[5,6] Obesity is a risk factor for cardiovascular disorders and metabolic diseases, such as coronary heart disease, hypertension, atherosclerosis, and diabetes mellitus.[7] Eighty percent of patients with type 2 diabetes mellitus in children are overweight children, while 60-90% are obese. A diagnosis of diabetes mellitus can be confirmed by examination of fasting blood glucose and the oral

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glucose tolerance test (OGTT). Alternatively, the *American Diabetes Association* (ADA), *International Diabetes Federation* (IDF), *European Association for the Study of Diabetes* (EASD), and *New Zealand Society for the Study of Diabetes* (NZSSD) recommended a glycosylated hemoglobin (HbAlc) cut-off point > 6.5% for diagnosing diabetes mellitus.[8] HbAlc examination is a blood test to evaluate blood sugar control, as it gives an average blood sugar over a period of 6-12 weeks. In a cohort study involving obese children and adolescents of various ethnicities, 21% of children and adolescents had HbA1c levels of 5.7-6.4%, and 1% had HbA1c levels > 6.5%, putting them at risk for diabetes.[9]

Waist circumference is an anthropometric measurement widely used to predict cardiometabolic syndrome in obese children and adolescents.[10] Another anthropometric indicator is the waist circumference to height ratio.[11,12] The waist-to-height ratio can be used to estimate body fat distribution.[13,14] In adults, central obesity has been associated with HbAlc levels.[15] However, waist circumference and waist-to-height ratio have not been widely used in children and adolescents as predictors for cardiovascular disorders and metabolic diseases in connection with HbAlc levels. The purpose of this study was to assess for possible correlations between HbA1c level and waist circumference and waist-to-height ratio, as well as to evaluate potential factors associated with HbA1c levels in overweight and obese adolescents.

Methods

This cross-sectional study was done in overweight and obese adolescents who were junior high school students in Yogyakarta in November-December 2013. The study was conducted in junior high school students because no such study had been conducted in adolescents, and obesity in adolescence is likely to continue into adulthood.

Inclusion criteria were junior high school students aged 12-15 years who met the criteria for overweight and obesity (BMI Z-score>+1 SD), were generally healthy, and willing to participate in the study. Subjects'parents provided signed informed consents. Exclusion criteria were students with congenital heart defects, kidney problems, and other severe medical conditions, taking long-term steroid therapy, or were not present during study data retrieval.

Waist circumference measurements were done with subjects in an upright position, feet 25-30 cm apart, without shoes, and the researcher located at the subject's side. We measured subjects'waist circumferences at the midpoint between the peak of the illiacal crest and the lower edge of the last rib, in an axillary midline.[16] Waist-to-height ratio was obtained by dividing the waist circumference (in centimeters) by height (in centimeters).[17] Subjects provided 5 mL blood specimens for examination of HbA1c levels by high-performance liquid chromatography (HPLC) in a private laboratory.

To analyze for correlations between HbAlc level and waist circumference as well as waist-to-height ratio, we used Spearman's correlation test. Multivariate analysis with linear regression test was used to assess variables potentially associated with HbA1c levels. This study was approved by the Medical Ethics Committee of Universitas Gadjah Mada Medical School, Yogyakarta, Central Java, Indonesia.

Results

Four junior high schools in Yogyakarta were randomly selected by cluster sampling: *Bopkri 5, IT Abu Bakar, SMP Negeri 3*, and *SMP Muhammadiyah*. Of 405 students initially screened, 93 students were overweight and obese (23%). Of these 93 children, 26 were excluded because they did not complete the examinations (23 children), had a history of serious illness (1 child), or had steroid treatment (2 children). The study profile is shown in **Figure 1**.

We included 67 subjects, comprising 48 girls (72%) and 19 boys (28%). Their age range was 12-15 years, with a mean age of 13.5 (95%CI 13.28 to 13.77) years. Forty-two subjects (63%) were overweight and 25 subjects (37%) were obese. Subjects' mean HbA1c was 5.6% (95%CI 5.49 to 5.65).

Normalization of Kolmogorov-Smirnov test revealed that waist circumference and waist-to-height ratio data were not normally distributed. Hence, we used Spearman's correlation test to analyze for associations. We found that waist circumference did not have any correlation with HbA1c level (r=0.18; P=0.15) in our overweight and obese subjects. However, waist-to-height ratio had a weak significant

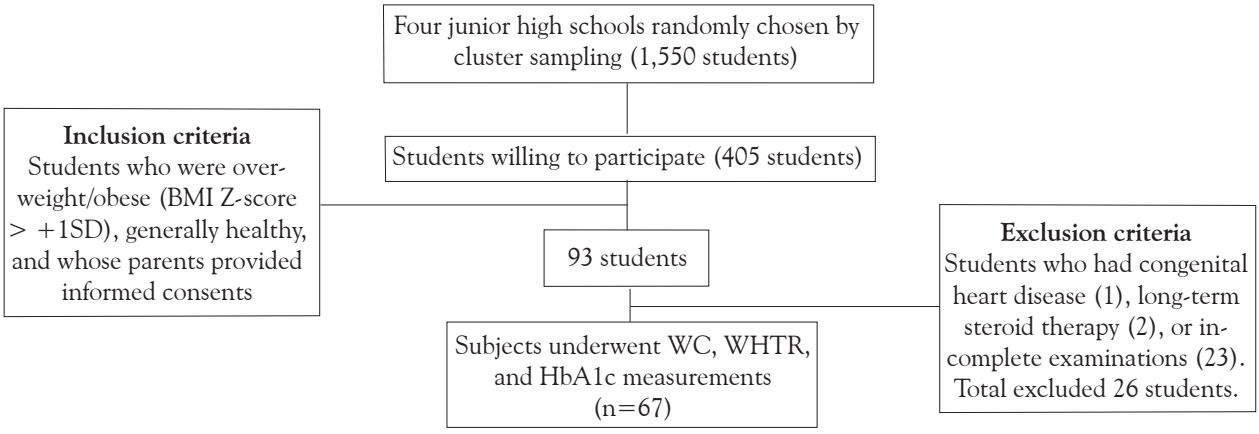

Figure 1. The course of study.
WC=waist circumference; WHTR=waist-to-height ratio

correlation with HbA1c levels (r=0.21; P=0.04) (**Table 1, Figure 2**).

We did linear regression analysis between independent variables and dependent variable levels of HbA1c. On univariate analysis we found that variables that had a value of P <0.25 were age, nutritional status, waist circumference, and waist-to-height ratio. Multivariate analyses were done to assess for associations between HbA1c level and other factors. The waist-to-height ratio was the only variable significantly associated with HbA1c level (P=0.02), while age, sex, and nutritional status had no effect on HbA1c levels (P values were 0.10, 0.34, and 0.45, respectively) (**Table 2**).

Table 1.HbA1c level,waist circumference, and waist-to-height ratio in overweight and obese adolescents

N=67	HbA1c level	P value
Waist circumference	r=0.17	0.18
Waist-to-height ratio	r=0.21	0.04

Discussion

The prevalences of overweight and obesity in junior high school adolescents in Yogyakarta were 16.8% and 6.2%, respectively. These percentages were higher

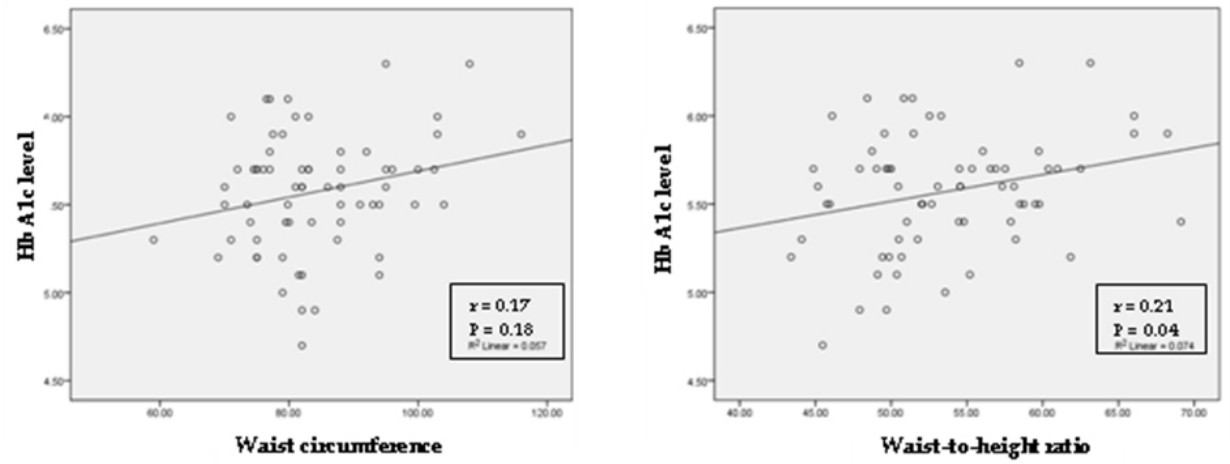

Figure 2. Scatter plots of HbA1c level, waist circumference, and waist-to-height ratio in overweight and obese adolescents

Table 2. Univariate and multivariate linear regression analyses of variables with potential associations with HbA1c levels in overweight and obese adolescents

Variables	Univariate linear regression		Multivariate linear regression	
	ß (95%CI)	P value	ß (95%CI)	P value
Age, year	-0.19 (-0.14 to 0.02)	0.13	-0.19 (-0.14 to 0.01)	0.10
Gender (1=boy, 2=girl)	0.12 (-0.09 to 0.27)	0.34	(-0.14 to 0.01)	
Nutritional status (1=overweight, 2=obese)	0.16 (-0.06 to 0.27)	0.20	-0.14 (-0.34 to 0.15)	0.45
Waist-to-height ratio	0.02 (0.00 to 0.03)	0.03	0.28 (0.00 to 0.03)	0.02

than that of a 2004 study that reported the percentage of obese adolescents in Yogyakarta to be 5.0%.[18]

Obesity may be due to lifestyle habits, such as eating high-fat foods and a lack of physical activity. Weight gain in children and adolescents is an important issue because it can continue into adulthood increasing the risk of cardiovascular diseases and metabolic disorders, such as diabetes.[19] Obesity can lead to the occurrence of type 2 diabetes, through an insulin resistance mechanism, namely, decreased insulin sensitivity which results in excessive insulin secretion by pancreatic ß-cells followed by hyperinsulinemia to maintain fasting blood glucose levels in the normal range. Beyond a certain point, compensating pancreatic ß-cells fail, causing hyperglycemia.[20,21] Central obesity is described as excessive fat deposits in the abdominal area, either subcutaneous fatty tissue or visceral adipose tissue rich in free fatty acids.[22] Therefore, cardiovascular diseases and metabolic disorders such as diabetes mellitus are more closely associated with central obesity than peripheral obesity.[23] Central obesity can be determined by waist circumference measurements > 90th percentile for age, and waist-to-height ratio ≥ 0.5.

We found no correlation between waist circumference and HbA1c level. In contrast, a Malaysian study reported that waist circumference was the only parameter associated with HbA1c levels, and not waist-to-height ratio or body mass index.[24] Measurement of waist circumference is simple to perform and has been shown to accurately detect the accumulation of abdominal fat, as compared to waist-to-hip ratio or body mass index measurements. Waist circumference alone was significantly more efficient for predicting insulin resistance, increased blood pressure, as well as increased serum cholesterol and triglyceride levels rather than body mass index. In children and adolescents, waist circumference > 90th percentile was associated with elevated insulin and lipid profiles, which are risk factors for cardiovascular disease and metabolic disorders.[25] However, our findings were consistent with data from previous studies, in that waist circumference and BMI were not significantly associated with HbA1c values, after controlling for age, race, sex, and height.[26] Since waist circumference does not take into account an individual's height, it has limited value for use in populations with wide varieties in heights, such as Indonesia.[27]

The waist-to-height ratio is an anthropometric index that can be used to easily detect visceral obesity and its association with cardiovascular diseases and metabolic disorders. The ratio is accurate for determining body fat, as it takes into account not only abdominal fat, but the percentage of muscle and waist circumference corrected by height, of each individual.[27] In our study, the waist-to-height ratio was significantly and positively correlated with HbA1c levels in overweight and obese adolescents. To date, no study reports have linked these two variables. But an earlier study in Yogyakarta stated that subjects with central obesity had a 1.21 times (95%CI 0.98 to 2.94) risk of impaired fasting glucose compared to a non-central obese group.[28] In contrast, a Malaysian study reported that waist-to-height ratio was not superior to waist circumference or BMI for predicting glycemic control in diabetes mellitus patients. This finding may be due to the fact that all study subjects, namely diabetes mellitus patients, regularly took medication including insulin, which could play a role in glycemic control. Such an anabolic effect may result in weight gain, increased appetite, and reduced glycosuria, with the end result of calorie retention.[24,29] Although not connected specifically with HbA1c

levels, previous studies in the United States, Japan, and some European countries showed a strong correlation between waist-to-height ratio and the risk of cardiovascular and metabolic diseases in overweight and obese adolescents. Hence, waist-to-height ratio has been proposed as an alternative measurement of central obesity in children.[14, 30]

Our multivariate analysis revealed that waist-to-height ratio was the only variable associated with HbA1c level (P=0.02), whereas age, sex, and nutritional status had no significant associations with HbA1c levels. Several previous studies stated that the waist-to-height ratio was the best measurement to determine central obesity and predict metabolic risks, because the waist-to-height ratio is a consistent value, as it is not influenced by differences in ethnicity and sex.[12] Furthermore, a previous study found that the prevalence of impaired glucose tolerance was significantly higher in the central obesity group compared to the non-central obesity group. Central obesity was the only risk factor for impaired glucose tolerance in obese adolescent girls in Yogyakarta with an OR of 4.6.[31] The use of body mass index (BMI) is not optimal for measuring the amount of fat tissue in children because it is influenced by age and race. In addition, high BMI cannot distinguish between excess fat or high muscle mass.[26,32]

In conclusion, waist-to-height ratio significantly associates with HbA1c level in overweight and obese adolescents.

Conflict of interest

None declared.

Acknowledgements

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Original Article

Risk factors for miliary tuberculosis in children

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Abstract

Background Miliary tuberculosis (TB) is a fatal form of tuberculosis with severe clinical symptoms and complications. The mortality rate from this disease remains high, therefore, it is important to identify the risk factors for miliary TB for early detection and treatment.

Objective To identify risk factors for miliary tuberculosis in children.

Methods A case-control study of children aged 0-14 years with miliary TB was conducted in Dr. Soetomo Hospital from 2010 to 2015. Data were taken from medical records. Case subjects were children with miliary TB, and control subjects were children with pulmonary TB. Patients with incomplete medical records were excluded. Case subjects were identified from the total patient population; control subjects were included by purposive sampling, with case:control ratio of 1:1. Potential risk factors were age, nutritional status, BCG immunization status, and history of contact with TB patients. Statistical analyses were done with Chi-square and logistic regression tests. P values < 0.05 were considered to be statistically significant.

Results A total of 72 children were analyzed, with 36 case and 36 control subjects. Nutritional status had a significant associatation with miliary TB in children (OR 3.182; 95%CI 1.206 to 8.398; P=0.018) in both bivariate and multivariate analyses. The probability of a child with moderate or severe undernutrition developing miliary TB was 76.09%. Other factors were not significantly associated with miliary TB.

Conclusion Nutritional status is significantly associated with miliary TB in children, and moderate or severe undernutrition increases the risk for developing miliary TB. **[Paediatr Indones. 2017;57:63-6. doi: http://dx.doi.org/10.14238/pi57.2.2017.63-6]**.

Keywords: miliary tuberculosis; children; risk factors

Miliary tuberculosis (TB) is caused by hematogenous and lymphatogenous dissemination of *Mycobacterium tuberculosis* bacteria in the body, infecting multiple organs. It accounts for 3–7% of all TB cases.[1] Although there have been few reports on the prevalence of miliary TB in Indonesian children, the Indonesian Ministry of Health reported 1,168 cases of pediatric pulmonary acid-fast bacilli (AFB) positive TB in 2014.[2] The mortality rate from miliary TB is usually around 25%, but may reach 100% if left untreated.[3]

Complications from the disease include respiratory distress syndrome, renal failure, pericarditis, shock, disseminated intravascular coagulation, and acute respiratory failure.[3,4] Miliary TB in children has been closely linked to the pathogenesis of TB meningitis (TBM), the most fatal form of TB. The proportion of children with miliary TB who suffer TBM is larger than that of adults with miliary TB.[4,5]

Some proposed risk factors for developing miliary TB in children have been younger age, malnutrition, lack of BCG immunization, and history of contact with tuberculosis patients.[4,6-8] Since pediatric TB and the severe complications of miliary TB are a health

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problem in Indonesia, we aimed to identify risk factors for miliary TB in children, in order to facilitate early prevention and intervention.

Methods

We conducted a hospital-based, case-control, retrospective study using secondary data from medical records of pediatric patients admitted to the Division of Respirology, Department of Child Health, Airlangga University Medical School, Dr. Soetomo Hospital, Surabaya from 2010 to 2015. This study was approved by the Medical Ethics Committee of Dr. Soetomo Hospital, Surabaya, East Java, Indonesia.

Subjects were divided into case and control groups. Children under 14 years of age and diagnosed with miliary TB were selected as case subjects. Children under 14 years of age and diagnosed with pulmonary TB using Indonesian Pediatric Tuberculosis Scoring System1 with a diagnostic score ≥6 were selected as control subjects. The scoring system was used only for the control subjects. Children with incomplete medical records were excluded. Cases were taken from the total population of those with miliary TB, while controls were included by purposive sampling, with a case: control ratio of 1:1.

Potential risk factors analyzed were age, nutritional status, BCG immunization status, and history of contact with TB patients. Age was categorized as ≤2 years or >2 years. Nutritional status was determined using the weight/height WHO curves for subjects under 5 years or the CDC curves for those over 5 years,[1] according to subject's sex. All data were taken from the medical records. We performed bivariate Chi-square and multivariate logistic regression analyses using *SPSS version 20* software. Results with P values <0.05 were considered to be statistically significant, with 95% confidence intervals.

Results

Out of 1,184 TB patients admitted during the study period, 46 had miliary TB. Ten patients were excluded, leaving 36 subjects in the case group. Thirty-six pulmonary TB patients were included as the control group. The characteristics of the subjects are presented in **Table 1**.

Table 1. Characteristics of subjects

Characteristics	N=72
Age, n(%)	
< 2 years	25 (34.7)
≥ 2 years	47 (65.3)
Nutritional status, n(%)	
Moderate or severe undernutrition	40 (55.6)
Normal	32 (44.4)
BCG immunization status, n(%)	
No	12 (16.7)
Yes	60 (83.3)
History of contact with TB patients, n(%)	
Yes	53 (73.6)
No	19 (26.4)

Bivariate analysis of the possible risk factors showed that only nutritional status was significantly associated with miliary TB in children (OR 3.182; 95%CI 1.206 to 8.398; P=0.018). Age, BCG

Table 2. Bivariate analysis of miliary tuberculosis risk factors in children

Variables	Case (n=36)	Control (n=36)	OR	95%CI	P value
Age, n					
< 2 years	16	9	2.400	0.882 to 6.528	0.083
≥ 2 years	20	27			
Nutritional status, n					
Moderate or severe undernutrition	25	15	3.182	1.206 to 8.398	0.018
Normal	11	21			
BCG immunization status, n					
No	9	3	3.667	0.902 to 14.901	0.058
Yes	27	33			
History of contact with TB patients, n					
Yes	25	28	0.649	0.225 to 1.871	0.422
No	11	8			

immunization status, and history of contact with associated with miliary TB in children (OR 3.182; 95%CI 1.206 to 8.398; P=0.018). Age, BCG immunization status, and history of contact with TB patients were not significantly associated with miliary TB (**Table 2**).

Multivariate analysis revealed similar results, with nutritional status as the only significant risk factor of miliary TB in children (**Table 3**). The probability of a child with moderate or severe undernutrition developing miliary TB was found to be 76.09%.

Table 3. Multivariate analysis of miliary risk factors in children

Variables	Coefficient (B)	OR	95%CI	P value
Age	0.702	2.017	0.653 to 6.235	0.223
Nutritional status	1.165	3.204	1.166 to 8.805	0.024
BCG immunization status	0.891	2.437	0.527 to 11.263	0.254

Discussion

We identified moderate/severe malnutrition as a significant risk factor for miliary TB in children. Similarly, a study in India found that malnutrition was a risk factor for miliary tuberculosis.[4] Also, another study in India found a significant association between nutritional status and the incidence of pulmonary tuberculosis infections.[9] A previous study in Peru also found a significant association between malnutrition and mortality in children with TB.[10] Furthermore, a systematic review of studies in developed countries like the United States, Hong Kong, Finland, and Norway, found a consistent relationship between the incidence of TB and the body mass index (BMI) of the patients, with a 14% increased risk of TB for a decrease of one BMI unit.[11]

The relationship between nutritional status in children and miliary tuberculosis incidence may be explained by Jaganath *et al*. who suggested that nutrient deprivation may have a detrimental effect on Th1 cells, which act as an important component in cell-mediated immune system defense against miliary TB.[12] A previous study reported that cell-mediated immunity is a key factor in host defense mechanisms against the progression of TB infection to active TB disease.[13] Therefore, the compromised cellular immune system in children with undernutrition possibly increases the risk of developing miliary TB from active TB disease and TB infection.

We found no statistically significant associations between age, BCG immunization status, or history of contact with TB patients and the incidence of miliary TB in children. However, we suggest that younger age and negative BCG immunization status may also increase the risk of miliary TB, as reported by previous studies.[6,7]

The difference between our findings and those of previous studies may be due to several reasons. First, age in our study may have been affected by the higher prevalence of older pediatric TB patients. A previous study noted that even though an age under 2 years was a risk factor of miliary TB, most children with TB infection in endemic areas were older than 2 years, so there was a higher chance of more children over 2 years suffering from miliary TB.[6] Another study in England and Wales also found more miliary TB in older children, which indicated the possible reactivation of latent disease.[14] Thus, we suggest that miliary TB should be suspected in children of any age. Second, BCG immunization coverage was high in our study. According to a systematic review by Trunz *et al*., the estimated efficacy of BCG prevention of miliary TB reached 77%, but in Asian countries, there might be an overestimation of the number, due to the inclusion of studies of countries with higher immunization coverage than Asian countries but with lower risk of infection, while Asian countries have higher rate of infection and reinfection despite the high immunization coverage.[15] Moreover, Fine found that the protective effect of BCG was influenced by geographic location, as the protective effect declined in regions closer to the equator.[16] BCG immunization itself should continue to be administered as regulated, but we suggest continuing future study for a better vaccine. Third, the majority of our subjects reported a positive history of contact with TB patients, in contrast to previous studies with more subjects who had no history of contact with TB patients.[8,17]

Therefore, contact tracing is an important task, but the precise relationship between contact and miliary TB needs further study.

A limitation of this study was the use of secondary data which could create a bias in the information we received. We propose future studies with better methodologies, preferably with a prospective design.

In conclusion, moderate/severe malnutrition is a significant risk factor for miliary TB in children. Further study is needed to elucidate the pathogenic mechanism between undernutrition and miliary TB.

Conflict of interest

None declared.

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Original Article

Third trimester maternal 1,25-dihydroxyvitamin D and neonatal birth weight

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Abstract

Background The main cause of neonatal mortality is low birth weight. Active form of vitamin D (1,25-dihydroxyvitamin D) increase the efficiency of calcium and phosphorous absorption in intestinal. Deficiency 1,25-dihydroxyvitamin D in pregnant woman was hipothesized relates with low birth weight in neonate.

Objective To determine the relationship between maternal 1,25-dihydroxyvitamin D level and neonatal birth weight.

Methods This was an observational study with cohort design to 47 women in the third trimester pregnancy. This study was cond ucted on August to December 2014. Subjects were taken from Ibu dan Anak Hospital, Padang, West Sumatera. Maternal blood from antecubital vein was examined for 1,25-dihydroxyvitamin D concentration using enzyme-linked immunosorbent assay (ELISA). Neonatal birth weights were measured right after delivery. Data were analyzed by Pearson's correlation and linear regression tests.

Results A positive correlation was found between maternal 1,25-dihydroxyvitamin D level and neonatal birth weight (R=0.910; R2=0.821; P=0.000). The 1,25-dihydroxyvitamin D level had an 82.1% contribution to the baby's birth weight, while other factors not assessed in this study had less of an effect.

Conclusion There is positive correlation between maternal 1,25-dihydroxyvitamin D levels in the third trimester of pregnancy and neonatal birth weight. **[Paediatr Indones. 2017;57:67-9. doi: http://dx.doi.org/10.14238/pi57.2.2017.67-9]**.

Keywords: *1,25-dihydroxyvitamin D; low birth weight; maternal; neonatal*

Neonates with low birth weight have 40 times higher risk of perinatal and infant death than neonates with normal birth weight.[1] Low birth weight is defined as full term or preterm neonates with birth weight <2,500 grams. Nutritional deficiency also associated with neonatal birth weight.[2] Nutritional intake must be sufficient during pregnancy including vitamin D as a micronutrient. Sufficient vitamin D should be maintained during pregnancy because vitamin D affects fetal growth in the subsequent trimester. Vitamin D deficiency in pregnant women may have negative effects in the mother and fetus, such as pre-eclampsia, gestational diabetes, preterm delivery, fetal growth restriction, and spontaneous abortion.[3]

The two forms of vitamin D usually measured in blood are 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)2D] as the active metabolite of vitamin D.[4] There was not many study that have examined the association between maternal [1,25(OH)2D] level and neonatal birth weight because

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[1,25(OH)2D] provides no information about vitamin D status and it is often normal or elevated in vitamin D deficiency. However, [1,25(OH)2D] increases the efficiency of calcium and phosphor absorption in intestinal.[5] Furthermore, [1,25(OH)2D] involves in placental cell maintenance, such as proliferation, differentiation, apoptosis, and also plays role in calcium homeostasis in the bones.[3] Deficiency of [1,25(OH)2D] in pregnant women will reduces the calcium absorption that will affect the embryo growth and development.[6] This study was conducted to determine relationship between maternal 1,25-dihydroxyvitamin D level and neonatal birth weight.

Methods

This was an observational study with cohort design that conducted on August to December 2014. The subjects were women in their third trimester of pregnancy that intend to deliver in Ibu dan Anak Hospital, Padang, West Sumatera, Indonesia. The inclusion criteria were pregnant women at >28 weeks of pregnancy, 20-35 year old, had parity more than three times, and agreed to participate in the study. Pregnant women with anemia, diabetes mellitus, kidney disorders, chronic hypertension, signs of clinical infection, twin pregnancy, history of preeclampsia or eclampsia, and did not deliver in Padang were excluded. The subjects were selected by consecutive sampling method.

This study was approved by the Committee of Medical Research Ethics of Dr. M. Djamil Hospital. Subjects were provided written informed consent and interviewed for their characteristics and pregnancy history. Subjects' blood was taken from antecubital vein for measure [1,25(OH)2D] levels. The blood samples were centrifuged at Dr. M. Djamil Hospital to obtain the serum and then brought to Biomedical Laboratory of Medical Faculty of Andalas University for ELISA assay. All subjects were followed up until delivery and the birth weight of the neonate was measured. Normality test was analyzed by Shapiro-Wilk test, followed by Pearson's correlation and linear regression tests.

Results

During the study period, we collected blood samples from 61 women, nine blood samples were excluded from the study because of low hemoglobin (<10 g/dL) (5 samples), leukocytes >17,000/mm3 (3 samples), and glucose level >149 mg/dL (2 samples). At the time of delivery, 5 subjects were also excluded because preterm delivery. Total 47 subjects were participated in this study.

As shown in **Table 1**, the mean concentration of [1,25(OH)2D] was 38.51 (SD 11.68) pg/mL. Two subjects had an extremely low concentration of [1,25(OH)2D] (9.47 pg/mL and 13.97 pg/mL). The mean neonatal birth weight was 2,963 (SD 404.5) grams.

Table 1. Mean 1,25-dihydroxyvitamin D concentration and neonatal birth weight

Variables	Mean (SD)	Range
1,25-dihydroxyvitamin D, pg/mL	38.51 (11.68)	9.47-66.55
Neonatal birth weight, g	2,963 (404.55)	2,200-4,200

Linear regression test revealed a positive correlation between [1,25(OH)2D] concentration and neonatal birth weight (R=0.910; R2=0.827; P=0.000), with a neonatal birth weight regression equation of 1,751.704+31.479 x 1,25-dihydroxyvitamin D. The [1,25(OH)2D] had contribution of 82.7% to neonatal birth weight, while the other 17.3% was contributed from other factors not investigated in this study.

Discussion

In this study, the mean maternal [1,25(OH)2D] concentration was 38.51 pg/mL, which was lower than the normal range in the third trimester pregnancy (60-119 pg/mL).[7] Bouillon *et al.* found the mean concentration of [1,25(OH)2D] in third trimester pregnancy was 97 pg/mL.[8] In other side, Kumar *et al.* found the [1,25(OH)2D] concentration of pregnant women in their third trimester was 39.5 pg/mL.[9] These inconsistent results maybe resulted from the difference of demographics, climate, or other variables that affect the [1,25(OH)2D] concentration, such as urea, creatinine, glucose, or leukocyte concentration. In our study, urea, creatinine, glucose, and leukocyte concentrations were normal in all subjects.

We found a strong correlation between [1,25(OH)2D] concentration and neonatal birth

weight, maternal [1,25(OH)2D] found contributed by 82.7% to neonatal birth weight. The [1,25(OH)2D] plays role in placental cell proliferation, differentiation, apoptosis, and the maintainence of calcium homeostasis in the bones, that affect trophoblast and spiral artery development. Moreover, calcium homeostasis can improve fetal bone growth. So, maternal [1,25(OH)2D] affects neonatal birth weight. To the best of our knowledge, our study was the first to examine the association between maternal [1,25(OH)2D] and neonatal birth weight.[6] A previous study found an elevated neonatal serum [1,25(OH)2D] concentration in very low birth weight infants, but it was hipothesized as a consequence of calcium and phosphor deficiency in the diet. The increase serum [1,25(OH)2D] act as a compensatory mechanism to increase intestinal calcium and phosphor concentration. Deficiency of [1,25(OH)2D] in pregnant women can result in maternal hypocalcemia, neonatal tetany, low birth weight, and rickets.[11] Vitamin D status during pregnancy plays an important role in fetal skeleton development, enamel formation, fetal growth, and development. The recent study showed that [1,25(OH)2D] regulates the secretion of the placental hormones, estradiol and progesterone, and suppresses inflammatory cytokines that can stimulate preeclampsia, premature delivery, and low birth weight.[12] In conclusion, maternal [1,25(OH)2D] concentration in the third trimester of pregnancy has a positive correlation with neonatal birth weight.

Conflict of Interest

None declared.

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Original Article

A hematologic scoring system and C-reactive protein compared to blood cultures for diagnosing bacterial neonatal sepsis

Deddy Eka Febri Liestiadi, Emil Azlin, Selvi Nafianti

Abstract

Background Neonatal sepsis is the leading cause of death after pneumonia. Definitive bacterial sepsis diagnoses are made by blood culture results, which require a lengthy time. C-reactive protein (CRP) levels and a hematologic scoring system by Rodwell *et al.* are rapid tests that may be useful for diagnosing neonatal sepsis.

Objective To determine the diagnostic value of CRP measurement and a hematologic scoring system compared to blood culture as the gold standard for diagnosing neonatal sepsis.

Methods A cross-sectional study was conducted from April to August 2015 in the Neonatology Ward of Haji Adam Malik Hospital, Medan. A total of 43 neonates who were clinically suspected to have sepsis underwent CRP, hematologic scoring, and blood cultures. The IT ratio and procalcitonin indices were also examined. Diagnostic values were analyzed by a 2x2 table.

Results Fourteen percent from all sample had positive bacterial culture. The CRP measurements had a sensitivity of 92.8%, specificity of 62%, positive predictive value (PPV) of 54.1%, negative predictive value (NPV) of 94.7%, positive likelihood ratio (PLR) of 2.44, and negative likelihood ratio (NLR) of 0.11. The hematologic scoring system had a sensitivity of 100%, specificity of 82.7%, PPV of 73.6%, NPV of 100%, PLR of 5.78, and NLR of 0. Procalcitonin and IT ratio show a good value of sensitivity and NPV, respectively.

Conclusion The hematologic scoring system has better specificity than CRP measurement as compared to blood culture. However, both tests have good sensitivity for diagnosing neonatal sepsis.

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Keywords: *C-reactive protein; hematologic scoring system; neonatal sepsis*

Neonatal sepsis is a clinical syndrome in the first 4 weeks of life, with signs of systemic infection and diagnosed by positive blood cultures.[1] Neonatal sepsis is life-threatening, particularly in developing countries, and is the second leading cause of death in neonates after prematurity.[2] Neonates should be protected from bacterial infections to prevent sepsis. The incidence of neonatal sepsis in Indonesia was reported to range from 20.7% to 38.7%.[3]

Etiologies of bacterial neonatal sepsis were identified over a 5-year span with the most common as follows: year 1998: *Klebsiella pneumoniae* (23%); 1999 and 2000: *Staphylococcus aureus* (17% and 6%, respectively); 2001 and 2002: Acinetobacter (6.7% and 20.4%, respectively); and 2003: *Klebsiella*

This study was presented at the *Pertemuan Ilmiah Tahunan Ilmu Kesehatan Anak VIII/PIT IKA VIII* (The 8th Child Health Annual Scientific Meeting), "Improving Professional Competence for Pediatrics Best Practice", Makassar, South Sulawesi, September 17-21, 2016.

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pneumoniae (23.4%).[4] At present, neonatal sepsis is classified based on pathophysiology to early-onset sepsis (EOS) and late-onset sepsis (LOS).[5]

A definitive neonatal sepsis diagnosis is established from blood cultures.[1] However, cultures require several days to allow for bacterial growth in culture medium. In central referral hospitals, procalcitonin and CRP examinations can help in diagnosing neonatal sepsis,[6,7] but these examinations cannot be performed in primary health care facilities. As such, several approaches, including a scoring system, may be implemented to simplify the diagnosis of neonatal sepsis.

A US study introduced a hematologic scoring system for diagnosing neonatal sepsis.[8] The scoring system consists of 7 findings, each of which are assigned a score of abnormal white blood cell count, abnormal total neutrophil [polymorphonuclear cells (PMN)] count, elevated immature to total PMN ratio, immature to mature neutrophil (IM) ratio > 3, elevated immature PMN count, platelet count < 150,000/mm^3, and degenerative changes in PMNs.[8,9] C-reactive protein has also been used to diagnose sepsis. The combination of CRP measurement and a hematologic scoring system is expected to be a new modality for rapidly diagnosing neonatal sepsis.

The aim of our study was to to determine the diagnostic value of CRP measurement and a hematologic scoring system compared to blood culture as the gold standard for diagnosing neonatal sepsis.

Methods

This cross-sectional study was done to determine the diagnostic value of CRP measurement and a hematologic scoring system compared to blood cultures, the current gold standard for bacterial neonatal sepsis. This study was held in the Neonatology Unit of, Haji Adam Malik Hospital, Medan, North Sumatera, from April to August 2015. The inclusion criteria were neonates with unstable temperature, lethargy, decreased muscle tone, history of resuscitation, changes in skin color such as jaundice and/or mottled, feeding disturbances, focal infections, metabolic or cardiopulmonary abnormalities, prematurity, history of fetal distress, history of rupture of the membran >18 hours, multiple gestation, premature rupture of the membranes, preterm birth, and/or chorioamnionitis. Exclusion criteria were neonates who had undergone blood culture examination, were diagnosed with sepsis, had received antibiotics prior to the study or whose parents declined participation.

Peripheral blood smears, PMN count, procalcitonin, IT ratio, IM ratio, immature PMN count, and degenerative changes in PMN were measured. Complete blood count and CRP examinations were conducted in the Clinical Pathology Laboratory. Blood cultures were done in the Microbiology Department. Neonates with a hematologic cut-off score of <4 were classified as not having sepsis, and those with a score of ≥ 4 were classified as having sepsis.

We use that cut off point depend on Rodwell's study.[8] Rodwell had formated a number of bacterial infection marker to form a hematologic scoring system which are assigned a score of: abnormal white blood cell count (≤ 5000 /μL or ≥ 25000/μL at birth or ≥ 30000/μL at 12-24 hour or ≥ 21000/μL day 2 onward =1), abnormal total polymorphonuclear cells (PMN)] count (1800-5400 =0, No mature PMN seen= 2, Increase/decrease =1), elevated immature to total PMN ratio (IT ratio > 0.12 =1), immature to mature neutrophil (IM) ratio ≥0.3 (=1), elevated immature PMN count(>600 =1), platelet count < 150,000/mm^3(=1), and degenerative changes in PMNs (toxic granules or cytoplasmic vacuoles =1). CRP examination was done qualitatively with a reference as follows: CRP ≥10 mg/L was considered to have sepsis and CRP <10 mg/L was considered to not have sepsis.

All subjects' parents provided written informed consent. This study was approved by the Health Research Ethics Committee of the University of Sumatera Utara Medical School.

To determine the diagnostic value of CRP and the hematologic scoring system for neonatal sepsis, we used a 2x2 table to obtain sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR). The hematologic scoring system was analyzed by receiver-operator characteristic (ROC) curve and measurement of the area under the curve (AUC) to determine the cut-off point.

Results

Forty-three neonates fulfilled the inclusion criteria. The clinical manifestations found in neonates who

participated in this study were: cardiopulmonary abnormalitites (58.1%), metabolic abnormalitites (11.6%), lethargy (7%), changes in skin color (9.3%), temperature changes (9.3%), and focal infections (4.7%). More than half of the neonates were female (55.8%) and had gestational age of <37 weeks (60.5%). The majority of neonates in this study had a chronological age of ≤72 hours (72.1%) and a birth weight of <2,500 grams (51.1%). At the end of the follow up, 14/43 (32.6%) of subjects had positive blood cultures. Positive blood cultures were found in 8/17 of neonates with gestational age of 37-42 weeks, and in 6/26 of neonates with gestational age of <37 weeks. Moreover, positive blood cultures were found in 6/31 of subjects with chronological age <72 hours, and in 8/12 of subjects with chronological age >72 hours. Positive blood cultures were observed in 12/21 of neonates with birth weight of 2,500 - 4,000 grams and in 2/22 of neonates with birth weight of <2,500 grams (**Table 1**).

A 2x2 table analysis revealed that the predictive value of the hematologic scoring system was more accurate than that of CRP measurement for diagnosing neonatal sepsis. The hematologic scoring system had sensitivity of 100% and specificity of 82.7%, while CRP had sensitivity of 92.8% and specificity of 62% (**Table 2**). In addition, the hematologic scoring system had an AUC of 94.6% with excellent statistical significance (**Figure 1**).

In addition to CRP and the hematologic scoring system, we compared procalcitonin and IT ratio to blood cultures as diagnostic modalities for neonatal

Table 1. Demographic characteristics of neonates

Characteristics	Blood culture		P value
	Positive (n=14)	Negative (n=29)	
Gender, n			
Male	7	12	0.837[a]
Female	7	17	
Gestational age, n			
< 37 weeks	6	20	0.191[a]
37-42 weeks	8	9	
Chronological age at admission, n			
≤ 72 hours	6	25	0.005[b]
> 72 hours	8	4	
Birth weight, n			0.002[a]
< 2,500 g	2	20	
2,500-4,000 g	12	9	

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

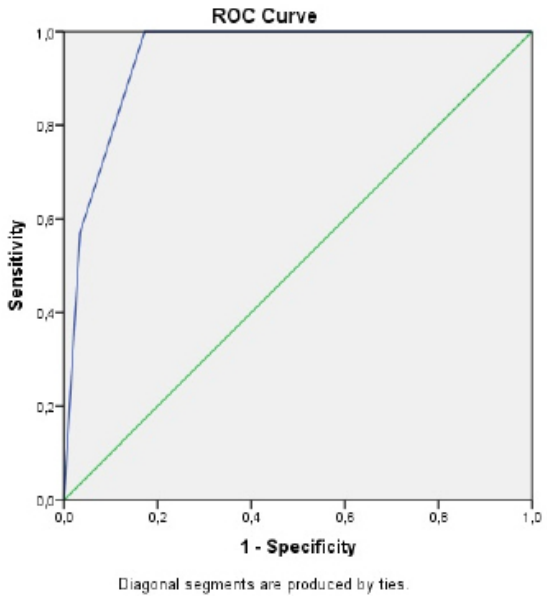

Figure 1. ROC of hematologic scoring system compared to blood cultures

Table 2. Diagnostic values of a hematologic scoring system and CRP compared to blood cultures

Diagnostic parameters	Blood culture		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR
	Positive (n=14)	Negative (n=29)						
Hematologic scoring system*, n								
Positive	14	5	100	82.7	73.6	100	5.78	0
Negative	0	24						
CRP*, n			92.8	62	54.1	94.7	2.44	0.11
Positive	13	11						
Negative	1	18						

*Diagnostic values were analyzed by a 2x2 table

sepsis. The IT ratio had sensitivity of 100% and specificity of 51.7%, while procalcitonin had sensitivity of 100% and specificity of 46.5% (**Table 3**).

Table 3. Diagnostic values of IT ratio and procalcitonin as compared to blood cultures

Diagnostic parameters	Blood culture		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR
	Positive (n=14)	Negative (n=29)						
IT ratio*, n								
Positive	14	14	100	51.7	50	100	2.07	0
Negative	0	15						
Procalcitonin*, n			100	46.5	33.3	100	1.86	0
Positive	14	28						
Negative	0	1						

*Diagnostic values were analyzed by a 2x2 table

protein measurement is an advanced examination commonly used to establish a diagnosis of sepsis. A previous study reported that the diagnostic value of

Discussion

Neonatal sepsis is a clinical syndrome in the first 4 weeks of life, with signs of systemic infection and diagnosed by positive blood culture results.[1] In developing countries, neonatal sepsis is the most frequent cause of death after prematurity.[2] A study in Haji Adam Malik Hospital, Medan from 2008 to 2010 found that the mortality rate from neonatal sepsis was 20%.[10] The prevalence of neonatal sepsis in Indonesia was 38.7% in 2005.3 In our study, the prevalence of neonatal sepsis was 33.7%.

The following bacteria were identfied from subjects' blood cultures: 3 *Acinetobacter baumannii*, 3 *Elizabethkingia meningoseptica* , 2 *Klebsiella pneumoniae*, 2 *Staphylococcus haemolyticus*, 1 Kocuria varians, 1 *Bacillus cereus*, 1 *Pseudomonas oryzihabitans*, 1 Salmonella spp , 1 P*asteurella pneumotropica*, and 1 *Flavimonas oryzihabitans*. A previous study also reported the most common etiology of neonatal sepsis to be Gram-negative bacteria.[11]

Bacteria entering the circulation trigger the body's immune response. The cell membrane and wall contain phosphocholine which can activate the complement system. An activated complement system induces granulocyte, phagocyte, and proinflammatory cytokine production.[12] C-reactive protein is an acute phase protein synthesized along with the activation of proinflammatory cytokines. The same thing will happen to granulocyte which is also a component of white blood cell.[12,13] C-reactive qualitative CRP measurement for neonatal sepsis had a sensitivity of 92.8% and specificity of 62%.[12] Another study involving a large sample size, found that sensitivity and specificity of CRP ranged from 29 to 100% and 6 to 100%, respectively.[14] The wide variability in diagnostic values of CRP may have been influenced by sample characteristics, study design, sample size, inclusion criteria, and differences in CRP cut-off points.[12] C-reactive protein levels increase 24 to 48 hours after clinical manifestations appear.[12] In our study, CRP examination was done according to chronological age of neonates at the time of admission to the Neonatology Unit. Chronological age of subjects ranged from 1 to 240 hours, with most neonates aged 2 hours (18.6%), 4 hours (11.6%), and 24 hours (11.6%). Given that many of our neonates were less than 24 hours old when their CRP was tested, we compared our sensitivity and specificity results to that from another study. A previous study found that the sensitivity of CRP in 24 hours after birth was 79% and the specificity was 78%.[15] The best time to measure CRP is between 24 and 48 hours after the onset of clinical manifestations.[12] Another study suggested measuring CRP after 6 to 12 hours.[16] Newborns' immature immune systems affect the amount of CRP detected in serum.[12]

Gestational age and birth weight also determine CRP level. Low gestational age neonates tend to have lower CRP levels compared to neonates with normal gestational age. A similar finding was observed with birth weight.[17] C-reactive protein levels increase at

a rate of 6% for each additional week of gestational age, and 2.4% for each 100 gram increase in birth weight.[12] We used 10 mg/L as the CRP cut-off point in accordance with previous studies which had ranges from 1.2 to 20 mg/L. The most common used value was 10 mg/L.[12,14,18] A single CRP examination is not considered to be representative due to the physiological changes in CRP level. Chiesa et al. determined cut-off points based on chronological age: 5 mg/L at birth, 14 mg/L at 24 hours, and 9.7 mg/L at 48 hours.[19] The same authors also determined cut-off points for full term and preterm neonates, with the highest levels of 11 mg/L and 13 mg/L, respectively.[20]

Neutropenia, thrombocytopenia, and leukopenia are indicators of severe infection and appear earlier than CRP.[20,21] Physiological changes in blood cells resulting from infection are components of the hematologic scoring system introduced by Rodwell *et al.*[8] this scoring system had sensitivity of 100% and specificity of 82.7% in our study, similar to the sensitivity of 80% and specificity of 90% in a previous study.[22] Leukopenia and neutropenia in neonatal sepsis were clearly observed until the first 3 days of life.[21] We also observed these conditions as the majority of our subjects' blood specimens were examined within 3 days of birth.

The IT ratio and procalcitonin were also measured in this study. The IT ratio had sensitivity of 100% and specificity of 51.7%, while procalcitonin had sensitivity of 100% and specificity of 46.5%. The IT ratio peaks from birth until 6 hours of life, but procalcitonin peaks at 12 hours or later after birth.[13] Vouloumanou et al. reported that procalcitonin had better diagnostic value in cases of late onset sepsis (LOS).[16] The timing of sample collection affected the diagnostic value of IT ratio, but not procalcitonin. Only 27.9% of subjects had procalcitonin levels in accordance with physiological changes.

A limitation of our study was the CRP diagnostic value determination, where variables were measured in a categorical scale. CRP was measured qualitatively, hence, the area under the ROC curve could not be calculated. This limitation influenced the cut-off point and further disrupted the prediction of neonatal sepsis. Furthermore, the non-uniform timing of blood specimen collections might have affected the results. The hematologic scoring system and CRP examinations should be done at 6 to 12 hours after birth, contrary to a previous study in which subjects underwent the examinations based on chronological age at admission.[16] The timing of antibiotic administration also affected the results. Antibiotic administration did not follow physiologic pattern of CRP and white blood cell activity and this might reduce the diagnostic value of CRP measurement.[16,21] In addition, the small sample size may limit the value of study. The NLR from the hematologic scoring system had a value of 0, which was caused by the presence of a cell in the 2x2 table with a value of 0. The strength of association in a comparative test between the hematologic scoring system and blood cultures could not be determined for the same reason.

In conclusion, the hematologic scoring system has better specificity than CRP, as compared to blood culture, for diagnosing neonatal sepsis. Nevertheless, both diagnostic tests have good sensitivity. A similar study with a larger sample size would be useful to confirm the diagnostic value of CRP and the hematologic scoring system.

Conflict of interest

None declared.

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Original Article

Hepatitis B seroprotection in children aged 10 – 15 years after completion of basic hepatitis B immunizations

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Abstract

Background The prevalence of hepatitis B viral (HBV) infection in Indonesia is high. The most effective way to control HBV infection is by hepatitis B (HB) immunization. Many studies reported that hepatitis B surface antibody (anti-HBs) seroprotection declines in children > 10 years of age. In addition many factors can influence anti-HBs titer.

Objective To measure anti-HBs titer and evaluate possible factors associated with anti-HBs titer.

Methods This cross sectional study was conducted in children 10-15 years of age from ten schools at Tuminting District, Manado, North Sulawesi, from October to November 2014. All subjects had completed the hepatitis B immunization scheme. By stratified random sampling, 105 children were selected as subjects. Data was analyzed with SPSS version 22.

Results From 48 schools, we selected 10 schools from which to draw a total of 105 children, but only 23 (21.9%) children had detectable anti-HBs . Of all subjects, 76 (72.4%) were female, 78 (74.3%) had good nutritional status, and 98 (93.3%) had birth weight ≥2,500 grams. Data from immunization record books showed that 26 (24.8%) subjects received the HB-1 vaccination at ≤7 days of age and 45 (42.9%) subjects had a ≥2 month interval between the HB-2 and HB-3 vaccinations. Multivariate analysis showed that administration of HB-1 at ≤7 days of age and a ≥2 month interval between HB-2 and HB-3 had significant associations with anti-HB seroprotection in children.

Conclusion A low proportion of subjects who had completed the hepatitis B immunization scheme had detectable anti-HBs titer (21.9%). Administration of HB-1 at ≤7 days of age and a ≥2-month interval between HB-2 and HB-3 vaccinations are important factors in anti-HB seroprotection in children aged 10-15 years. **[Paediatr Indones. 2017;57:76-83. doi: http://dx.doi.org/10.14238/pi57.2.2017.76-83]**.

Keywords: *seroprotection; anti-HBs titer; factors influencing anti-HBs titer*

Hepatitis B viral (HBV) infection is a global issue because it causes severe complications, such as liver cirrhosis, portal hypertension, and hepatocelluler carcinoma.[1] *The World Health Organization* (WHO) reported that approximately 900 million people are infected with hepatitis B (HB) and 378 million people are carriers. Each year, 620,000 people with hepatitis B die. Indonesia has been classified as a moderate- to high- prevalence area, with a mean of 9.4%, indicating that 1 in 10 of Indonesia's population have been infected with HBV.[2,3] In high endemic regions, infection often occurs at an early age and is transmitted vertically from mother to child or horizontally from chronic carriers who live in the same house.[4,5] Data from the *Indonesian Ministry of Health* in 2000 showed an HBV vertical transmission of 45.9% from mother to infant, mainly occurring at birth.[6] To date, the most effective way to control HBV infection is by HBV immunization. Although seroprotection titers decline with increasing age, the immune

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memory does not wane. Evidence of the above was found in a Thai study in children who were given the recombinant HB vaccine in infancy. Protective anti-HBs titers were found in 89.8% of subjects at the age of 5 years.[7] Given that the epidemiological pattern of HB in Indonesia is similar to that in Thailand, it can be concluded that booster immunizations at age of 5 years are not needed.[8] However, other studies showed that protective anti-HBs titers at the of age 10-12 years were only 12% - 47.9%.[9-15] In addition, the *Ministry of Health* figures in 2013 show coverage of the third dose of hepatitis B immunization in Indonesia is only 75.6%, which is still below the level of minimum protection (80%).[16]

Past studies have provided evidence that many factors can influence anti-HBs titers, such as age, sex, nutritional status, birth weight, administration of HB-1 at ≤ 7 days or >7 days, an interval between HB-2 and HB-3 of <2 months or ≥2 months, maternal age at delivery, maternal education level, and parental socioeconomic level.[9,13,17-23] Yet study on factors that influence anti-HBs titers are still rare. However, anti-HBs titers varied among studies, and, to our knowledge, a study of this type has never been conducted in Manado. As such, we aimed to evaluate anti-HBs titers and some possible associated factors.

Methods

This cross sectional study was conducted from October to November 2014 in children aged 10-15 years at the elementary, junior, and high schools in Tuminting District, Manado, North Sulawesi. Subjects were chosen randomly with stratified random sampling. Inclusion criteria in this study were: 1) children in good health, 2) had completed the HB basic immunization scheme (3 times) with an interval between HB-1 and HB-2 of 4-8 weeks and at a maximum of 12 months of age, 3) had approval from parents/guardians to participate in the study by signing a consent form following the study and approval of medical action, 4) had an health and immunization book/card (Kartu Menuju Sehat/KMS). Exclusion criteria were children with: 1) positive HBsAg, 2) malignant disease (leukemia, osteosarcoma, or lymphoma), chronic liver disease (cholestasis or HB), diabetes mellitus, diseases of immunodeficiency (HIV), chronic diseases (tuberculosis), chronic kidney disease requiring dialysis, hematology abnormalities requiring serial blood transfusion and immunosuppressant treatment use, or 3) a narcotic drug user (marijuana, morphine, or methamphetamine).

The minimum required sample size was estimated to be 97. This study was approved by the Research Ethics Committee of Sam Ratulangi University Medical School. Seroprotection status was classified as either an anti-HBs titer of ≥ 10 mIU/mL, categorized as responders (seroprotected), or an anti-HBs titers of <10 mIU/mL, categorized as non-responders. The responders were further categorized again as hyporesponder (anti-HBs titers 10-100 mIU/mL) or good responders (anti-HBs titers >100 mIU/mL). Data was analyzed with *SPSS version 22*. Bivariate data were analyzed using Chi-square and Mann Whitney test, followed by multivariate analysis, for results with $P<0.05$. Multivariate analysis was used to look for significance and regression coefficients. A P value <0.05 was considered to be statistically significant.

Results

This study was conducted in children aged 10-15 years at the elementary, junior, and high schools at Tuminting District, Manado, North Sulawesi. Of the 48 schools in the area, 105 study subjects were randomly chosen from 10 schools.

Characteristics of study subjects are shown in **Table 1**. Of the 23 seroprotected children, only two children had anti-HBs titers ≥ 100 mIU/mL. Two out of 105 subjects (1.9%) had mothers with a history of HBV infection. The 23 seroprotected subjects were further divided into groups based on the vaccination interval between HB-2 and HB-3, <2 months (2 subjects) and ≥2 months (21 subjects). Of the 21 subjects in the ≥2 month interval group, we found that the vaccination interval between HB-2 and HB-3 was 2 months for 10 subjects (mean anti-HBs titers 24.81 mIU/mL), 3 months for 7 subjects (mean anti-HBs titer 44.78 mIU/mL), 4 months for 1 subjects (anti-HBs titer 18.54 mIU/mL), 5 months for 1 subject (anti-HBs titer 51.27 mIU/mL), 6 months for 1 subject (anti-HBs titer 101.05 mIU/mL), and 8

Table 1. Study subjects characteristics based on age

Characteristics	Age (years)						Total N=105
	10 n=18	11 n=18	12 n=15	13 n=19	14 n=18	15 n=17	
Gender, n(%)							
Male	6	2	5	5	9	2	29 (27.6)
Female	12	16	10	14	9	15	76 (72.4)
Nutritional status, n(%)							
Undernutrition	7	7	1	5	4	3	27 (25.7)
Good nutrition	11	11	14	14	14	14	78 (74.3)
Birth weight, n(%)							
< 2,500 g	3	0	1	2	1	0	7 (6.7)
≥ 2,500 g	15	18	14	17	17	17	98 (93.3)
HB-1 administration, n(%)							
≤ 7 days	3	7	4	2	4	6	26 (24.8)
> 7 days	15	11	11	17	14	11	79 (75.2)
Interval between HB-2 and 3, n(%)							
< 2mo.	10	10	5	10	10	15	60 (57.1)
≥ 2 mo.	8	8	10	9	8	2	45 (42.9)
Maternal age at delivery, n(%)							
< 20 yrs	3	1	0	3	3	0	10 (9.5)
20-35 yrs	13	15	14	15	13	16	86 (81.9)
> 35 yrs	2	2	1	1	2	1	9 (8.6)
Maternal education, n(%)							
Elementary	2	3	2	1	0	0	8 (7.6)
Junior High	3	2	6	6	5	4	26 (24.8)
Senior High	12	12	7	10	12	11	64 (60.9)
College	1	1	0	2	1	2	7 (6.7)
Family income, n(%)							
< 2 million IDR/month	7	3	5	6	2	3	26 (24.8)
≥ 2 million IDR/month	11	15	10	13	16	14	79 (75.2)
Anti-HBs titer*, n(%)							
≥10 mIU/mL	5	5	4	2	4	3	23 (21.9)
< 10 mIU/mL	13	13	11	17	14	14	82 (78.1)

months for 1 subject (anti-HBs titer 48.84 mIU/mL) (data not shown).

Bivariate analysis of factors influencing anti-HBs titer are shown in **Table 2**. We found that children in the reactive group had better nutritional status compared to children in non-reactive group. Among subjects in non-reactive group, we found more children who received the Hb-1 vaccination at >7 days and the interval between Hb-2 and Hb-3 vaccination was < 2 months.

Multivariate analysis of factors influenced anti-HBs titer are shown in **Table 3**. We found the HB-1 vaccination at ≤7 days of age, and a ≥ 2 month interval between the HB-2 and HB-3 vaccinations were the significant factors that influencing anti-Hbs titer.

Discussion

We designed this study to be conducted on children aged 10-15 years, due to evidence contradictory to the *European Consensus Recommendations* (ECR) that HB seroprotection lasts for at least 10 years. Moreover, the ECR found that although anti-HBs titers decline considerably with age, even to negative values, a person is still clinically protected from chronic illness due to in vitro immunologic memory that still provides protection. As such, the booster is said to be no longer needed for people who received the complete basic HB immunizations.16 However, the reality on the ground shows that despite the HB immunization program having been implemented throughout Indonesia since 1997, the prevalence of HB infection

Table 2. Bivariate analysis of factors associated with anti-HBs titer

Characteristics	Anti-HBs titer		Relative risk (95%CI)	P value
	Non reactive n=82	Reactive n=23		
Child's age, n(%)				
10 years	13 (15.9)	5		0.159
11 years	13 (15.9)	5		
12 years	11 (13.4)	4		
13 years	17 (20.8)	2		
14 years	14 (17.0)	4		
15 years	14 (17.0)	3		
Mean age (SD), years	12.59 (1.71)	12.17 (1.78)		
Gender, n(%)				
Male	21 (25.6)	8	0.65	0.192
Female	61 (78.1)	15	(0.24 to 1.74)	
Nutritional status				
Under-nutrition	18 (21.9)	9	2.29	0.048
Good nutrition	64 (78.1)	14	(0.85 to 6.13)	
Birth weight, n(%)				
< 2,500 g	5 (6.1)	2	1.47	0.329
≥ 2,500 g	77 (93.9)	21	(0.27 to 8.10)	
HB-1 administration, n(%)				
≤ 7 days	14 (17.0)	12	1.47	0.329
> 7 days	68 (83.0)	11	(0.27 to 8.10)	
Interval between HB-2 and -3, n(%)				
< 2 months	58 (70.0)	2	5.3	0.001
≥ 2 months	24 (29.3)	21	(1.95 to 14.4)	
Socioeconomic level, n(%)				
< 2 million IDR/month	25 (30.5)	1	0.1	< 0.001
≥ 2 million IDR/month	57 (69.5)	22	(0.01 to 0.8)	
Maternal age at delivery, n(%)				
< 20 years	8 (9.8)	2		0.494
20-35 years	67 (81.7)	19		
> 35 years	7 (8.5)	2		
Maternal education level, n(%)				
Elementary	8 (9.8)	0		0.239
Junior High	20 (24.4)	6		
Senior High	49 (59.7)	15		
College	5 (6.1)	2		

Table 3. Multivariate analysis of factors influencing anti-HBs titer

Variables	Regression coefficient (B)	P value
Administration of HB-1 at ≤ 7 days or > 7 days	2.223	0.02
Interval between HB-2 and -3 distance < 2 mo. or ≥ 2 mo.	3.614*	< 0.001
Parental socioeconomic level	2.289*	0.96
Nutritional status	1.742	0.076

*=negative

in Indonesia remains high at 9.4%.[2,24] This evidence is also supported by the numbers of HB-0 immunization coverage in Indonesia at only 79.1% and DPT-HB-3 at 75.6%. Hence, the minimum standard of protection of 80% has not been reached.[16]

In addition to several previous studies, it is known that anti-HBs titers (seroprotection) decline in children with age (age ≥10 years), which varies from 12-47.9%.[9-12,14,15] Furthermore, Rathore found 24 subjects infected with HBV despite getting complete basic HB immunizations, so he suggested that the administration of a booster is needed to eradicate HB infection.[25]

In this study, titers of anti-HBs ≥ 10 mIU/mL were as follows: at age 10 years 5/18 (27.8%), age 11 years 5/18 (26.3%), age 12 years 4/15 (23.5%), age 13 years 2/19 (10.5%), age 14 years 4/18 (22.2%), and age 15 years was 3/17 (17.6%), with a mean total seroprotection at ages 10-15 years of 23/105 (21.9%).

The latest data indicate that protection after HB immunization did not last long term. However, we do not know whether the subjects in this study had formed anti-HBs that later declined, or did not have anti-HBs from the beginning, because we did not have subjects' initial data. Our findings regarding subjects with anti-HBs titers ≥ 10 mIU/mL were similar to those from other studies: Eldesoky *et al.*[11] at age >10 years were 33.3%, Lin *et al.*[14] at age > 12 years were 37.4%, Suraiyah *et al.*[15] at the age of 10-12 years were 38%, and Aswati *et al.*[9] at age 12 were 35%.

We found that 82 children had titers of anti-HBs <10 mIU/mL, while 23 children had seroprotection, with anti-HBs seroprotection titers ranging from 10.07 to 118.01 mIU/mL as well as the average of 36.45 (SD 29.21) mIU/mL (data not shown). Of the 23 seroprotective children, 21 children had anti-HBs titers <100 mIU/mL, while only 2 children had anti-HBs titers ≥ 100 mIU/mL (data not shown). Hence, the majority of children in this study (78.7%) were non-responders (anti-HBs titers <10mIU/mL); 21 children (19.4%) were hyporesponder; and only 2 children (1.9%) were good responder.22,26 We provided a booster for the 82 non-responders in accordance with the recommendation of the *Immunization Task Force of the Indonesian Pediatrics Society* (IPS).[27]

We also found that 3/105 children (2.8%) had mother with a history of HB infection, and from that, two children had titers <10 mIU/mL, but one child had anti-HBs titers ≥ 10 mIU/mL, so that the children with no seroprotection needed to be given HB booster immediately, because 45.9% HBV can be transmitted to the babies.[28,29]

We examined factors with potential to influence anti-HBs titers, such as age of the child at the time of examination of anti-HBs titers, sex, nutritional status, birth weight, administration of HB-1 at ≤ 7 days or >7 days, interval between HB-2 and HB-3 of <2 months or ≥2 months, maternal age at delivery, maternal education level, and parental socioeconomic level. Bivariate analysis of risk factors using Chi-square test revealed a significant relationship between seroprotection and administration of HB-1 ≤7 days, interval between HB-2 and HB-3 of ≥2 months, parental socioeconomic status, and child's good nutritional status. Further analysis with logistic regression revealed a highly significant positive correlation between seroprotection and interval between HB-2 and HB-3 of ≥2 months and in administration HB-1 ≤7 days, but no significant relationship between anti-HBs titers and parental socioeconomic level or nutritional status.

We observed a trend in declining anti-HBs titer with increasing age, though no statistically significant relationship was found. Similarity, Aswati *et al.* found no relationship between age and anti-HBs titers.9 In contrast, Whittle et al. found that anti-HBs titers were associated with age, where older children having lower titers (P<0.05).[30] We found no significant relationship between anti-HBs titers and gender, similar to a study by a previous study.[21] In contrast, studies in Iran and China showed that women had higher antibody responses than men.[31,32] In addition, another study showed a decline in the number of T-lymphocytes in males compared to females, with males having lower serum titers of IgM and IgG than females.[30] Differing immune responses in men and women were theorized be influenced by the sex steroid hormones, such as estrogen, progesterone, and testosterone.[33] We also found no correlation between anti-HBs titers and birth weight. This finding contrasts with the theory that low birth weight (LBW) and/or premature infants, have a low antibody titers due to passive immunity through maternal transmission, low complement level, macrophage function, and chemotactic response, as well as a lack of membrane deformability.[19] We found no relationship between the maternal age at delivery and anti-HBs titer, but there was a tendency that mother with lower educational level and age <20 years lacked knowledge and psychological readiness in child care, which may have affected the titer of anti-HBs.[34]

The immunization schedule had a significant positive relationship with anti-HBs titers, i.e., HB-1 administration at the age of ≤7 days and the interval between HB-2 and HB-3 of ≥2 months. A cross-sectional study by Mohammed et al. compared two immunization HB schedules and found that immune responses were similar in children who used an immunization schedule of 3, 4, and 9 months and one of 0, 2, and 9 months.[35] In addition, Damme et al. assessed anti-HBs titers 5 years after HB immunization, comparing two immunization schedules. Group A received HB vaccinations at 0 and 6 months, while group B received them at 0, 1, and 6 months. In the age group of 11-15 years, they observed anti-HBs titers of >10 mIU/mL in 79.5% of

group A and 91.4% of group B.[36]

Infants who received HB-1 immunization immediately after birth had higher seroprotection titer than other babies.[37] We found a significant association in seroprotected HB titers in infants immunized at ≤7 days compared to infants immunized at >7 days of age. However, when viewed from a number of children who are reactive after given the HB-1 ≤ 7 days in 12/23 children (52.2%) and non-reactive in 14/82 children (17.0%), this data shows there is almost no difference in the percentage and it demonstrates that the titer anti-HBs on the administration of HB-1 ≤ 7 day likely influenced by other factors.

The third dose is a determinant of antibody response as a booster dose, the longer the interval between the second and third immunizations (4-12 months), the higher the antibody titer.[37-39] We found a significant positive association between higher HB titers at an interval between HB- 2 and HB-3 immunizations of ≥ 2 months. However, when seen from the number of children who are reactive at interval of HB-2 and HB-3 immunization ≥ 2 months in 21/23 children and non-reactive in 24/82 children (29.3%), this data shows there is almost no difference in the percentage and this indicates that the anti-HBs titers at interval of HB-2 and HB-3 immunization ≥2 months likely influenced by other factors. Handayani *et al.*[40] reported a significant difference between administration of the first dose of HB vaccine at the age of ≤7 days and age> 7 days on anti-HBs titers (64.92 mIU/mL vs. 145.56 mIU/mL, respectively; P=0.038), but no relationship between HB-2 and HB-3 immunization interval and anti-HBs titers. Hepatitis B immunization at less than one week of age is intended to prevent vertical transmission from mother to baby, especially for women testing positive for HBsAg.

We found no correlation between anti-HBs titers and nutritional status of children in the multivariate analysis, although bivariate analysis show significant correlation. This result showing a nutritional status at the time of the study hence does not affect anti-HBs titers formation. A limitation of our study was that we did not obtain sufficient data to determine the subjects' nutritional status at the time they received basic HB immunizations. A Tanzanian study on children aged <5 years found 70.3% had good nutritional status, but there was no significant relationship between nutritional status and anti-HBs titers.[41] An Egyptian study examined the relationship between malnutrition and HB vaccine response in 27 infants with kwashiorkor or marasmus, and 13 healthy babies in the control group. Anti-HBs titers were significantly higher in the control group.[42]

A higher percentage of reactive subjects (95.7%) had higher parental socioeconomic status than non-reactive subjects (69.5%), but this finding was not significant on multivariate analysis. Ochirbat et al. found that children with socio-economically disadvantaged parents tended to lose seroprotection against HB compared to children of parents with good socioeconomic level.[17]

A limitation of this study was that we did not conducted HBsAg examintations on study subjects, therefore, we do not know if anti-HBs titers increased as a result of the subjects being naturally exposed to HB, or as a result of HBV immunization.

In conclusion, children aged 10-15 years have low seroprotection (21.9%) and administration of HB-1 at ≤7 days and an interval between HB-2 and HB-3 of ≥2 months are important factors associated with higher HB seroprotection. Based on our findings, we recommend giving booster to teenagers to increase the likelihood of their own seroprotection as well as decrease chances of future maternal-fetal transmission of HBV.

Conflict of Interest

None declared

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Original Article

Blood pressure-to-height ratio for diagnosing hypertension in adolescents

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Abstract

Background Diagnosing hypertension in children and adolescents is not always straightforward. The blood pressure-to-height ratio (BPHR) has been reported as a screening tool for diagnosing hypertension.

Objective To evaluate the diagnostic value of blood pressure-to-height ratio for evaluating hypertension in adolescents.

Methods A cross-sectional study was conducted among 432 healthy adolescents aged 12-17 years in Singkuang, North Sumatera from April to May 2016. Blood pressure tables from the National High Blood Pressure Education Program (NHBPEP) Working Group on High Blood Pressure in Children and Adolescents were used as our standard of comparison. Sex-specific systolic and diastolic blood pressure-to-height ratios (SBPHR and DBPHR) were calculated. ROC curve analyses were performed to assess the accuracy of BPHR for discriminating between hypertensive and non-hypertensive adolescents. Optimal thresholds of BPHR were determined and validated using 2x2 table analyses.

Results The accuracies of BPHR for diagnosing hypertension were > 90% (P<0.001), for both males and females. Optimal SBPHR and DBPHR thresholds for defining hypertension were 0.787 and 0.507 in boys, respectively, and 0.836 and 0.541 in girls, respectively. The sensitivities of SBPHR and DBPHR in both sexes were all >93%, and specificities in both sexes were all >81%. Positive predictive values for SBPHR and DBPHR were 38.7% and 45.2% in boys, respectively; and 55.9% and 42.4% in girls, respectively; negative predictive values in both sexes were all >97%, positive likelihood ratios in both sexes were all >5, and negative likelihood ratios in both sexes were all <1.

Conclusion Blood pressure-to-height ratio is a simple screening tool with high sensitivity and specificity for diagnosing hypertension in adolescents. **[Paediatr Indones. 2017;57:84-90. doi: http://dx.doi.org/10.14238/pi57.2.2017.84-90]**.

Keywords: *adolescents; blood pressure to height ratio; hypertension*

The prevalence of adolescent hypertension in developing countries is rising steeply.[1,2] Examination of the national childhood blood pressure data from several epidemiological studies in adolescents aged 13 to 15 years, according to the criteria of the *2004 Fourth Report from the National High Blood Pressure Education Program (NHBPEP) Working Group on Children and Adolescents*, indicated that 20% of adolescent boys and 13% of adolescent girls met the criteria for prehypertension. In adolescent subjects with BP measurements taken 2 years apart, the rate of progression from prehypertension to hypertension was 7% per year.[3,4] In 2013, the *Indonesian Ministry of Health* reported a 5.3% prevalence of hypertension in youth aged 15 to 17 years (6% for boys and 4.7% for girls), according to the JNC VII 2003 criteria.[5,6] However, a study found that 45.2% of 200 children with

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a family history of hypertension were hypertensive, in a study of high school students in Soposorung, North Sumatera.[7]

Hypertension in children and adolescents can lead to adult hypertension. Hypertension is a known risk factor for coronary artery disease in adults, and its presence in children and adolescents may contribute to the early development of coronary artery disease. Appropriate early-stage diagnosis and intervention in children and adolescents are important for reducing the risk of hypertension-related disorders in adults.[8,9] Identifying hypertension in children and adolescents is more complicated than in adults.[1,2] Diagnosis of hypertension in children and adolescents, according to the *NHBPEP Working Group on Children and Adolescents*, is influenced by age, sex, and height.[3] As a result, the threshold values of hypertension in children and adolescents vary according to these parameters. Hence, alternative, less cumbersome, diagnostic tools for adolescent high blood pressure are under evaluation.[1,2]

Several studies reported associations between blood pressure and weight, height, and age.[10,11] Height has an especially important role in determining blood pressure because body size affects blood pressure.[12-14] The hydrostatic paradox, first described by Stevin *et al.*, stipulates that fluid pressure generated at the bottom of a tube can be measured from the vertical height of the tube. Fluid can flow from the top to the bottom of the tube when the pressure at the bottom of the tube exceeds the hydrostatic pressure that is defined by its height.[15] Kahn *et al.* then applied this principle to a child's blood pressure, naming it the hydrostatic column of blood hypothesis. Adequate perfusion to the child's brain can be achieved when the blood pressure in the heart exceeds the hydrostatic pressure defined by the vertical distance between the heart and vertex.[12]

High blood pressure indirectly identified through anthropometric indicators may be an efficient strategy for detection and control.[16] In 2010, Lu *et al.* evaluated, for the first time, the feasibility and accuracy of the blood pressure-to-height ratio (BPHR) for identifying hypertension in Chinese adolescents.[2] The study was replicated in Nigerian adolescents by Ejike.[1] Both studies concluded that the BPHR is a simple method for diagnosing hypertension in children and adolescents.[1,2] Therefore, we evaluated the diagnostic value of BPHR for diagnosing hypertension in Indonesian adolescents.

Methods

This prospective, diagnostic study with cross-sectional design was conducted in healthy adolescents aged 12 to 17 years in Singkuang, Mandailing Natal District, North Sumatra from April to May 2016. Adolescents who had overt signs of ill health on physical examination or who related a history of being on medications for any diseases/conditions were excluded from the study. Subjects' parents or legal guardians provided written informed consent.

Subjects were collected by consecutive sampling and their ages were obtained from school records. Subjects underwent height measurements, in a standing position without shoes, using a portable stadiometer, to the nearest 0.1 cm. Before blood pressures were measured, subjects were asked to rest for an initial 10 minutes, in a seated position and in a quiet room. Three separate blood pressure readings were taken per subject, at five-minute intervals, using a mercury sphygmomanometer. Appropriate cuff sizes were used for each subject. Systolic blood pressure (SBP) was determined by the onset of the 'tapping' Korotkoff sounds (K1); diastolic blood pressure (DBP) was determined by the fifth Korotkoff sound (K5). Systolic blood pressure-to-height ratio (SBPHR) was calculated as SBP (mm Hg)/height (cm), and diastolic blood pressure-to-height ratio (DBPHR) was calculated as DBP (mm Hg)/height (cm).[1,2]

Blood pressure tables from *The Fourth Report from the NHBPEP Working Group on Children and Adolescents* (2004) were used as our standard of comparison to determine the subjects' hypertensive status. Normal blood pressure was defined as systolic and diastolic blood pressure $< 90^{th}$ percentile for gender, age, and height. Prehypertension was defined as systolic or diastolic blood pressure $\geq 90^{th}$ percentile but $< 95^{th}$ percentile for gender, age, and height. Hypertension was defined as systolic or diastolic blood pressure $\geq 95^{th}$ percentile for gender, age, and height.[3]

Numerical variables were reported as median (minimum – maximum). Comparisons were performed between groups using Mann-Whitney test. Spearman's correlation coefficient was used to measure the strength of association between two variables. Results with P values < 0.05 were considered to be statistically significant.[17] A receiver operating characteristic (ROC) curve was used to assess the discriminatory

ability/diagnostic accuracy of SBPHR and DBPHR (separately), with respect to normotensive and hypertensive subjects, as defined by the age-, gender-, and height-specific reference standards.[3] The ROC curves were plotted using measures of sensitivity and specificity for the various cut-off points. The area under the curve (AUC) is a measure of this discriminatory/diagnostic power of a test. An AUC value of 1.0 indicates a perfectly accurate test, whereas an AUC value of 0.5 indicates that the test performs worse than chance. Optimal cut-off points for both SBPHR and DBPHR were determined by specificity and sensitivity values that yielded the maximum sums from the ROC curves. Using the determined cut-off points as diagnostic tools, normotension and hypertension were defined in the population, and the sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio of the thresholds were calculated.[17] All data analyses were done using *SPSS 17.0* statistical software.

Results

A total of 450 adolescents, junior and senior high school students were initially screened for this study. Eleven adolescents were excluded because they did not meet the age criteria and 7 adolescents refused to participate. Of 432 subjects, 177 (40.9%) were boys and 255 (59.1%) were girls. All subjects were from the Batak Mandailings tribe, Mongoloid race.

The characteristics of study subjects are presented in **Table 1**. The age distribution of subjects was as follows: 28 (6.5%) adolescents aged 12 years, 88 (20.4%) adolescents aged 13 years, 79 (18.3%) adolescents aged 14 years, 96 (22.2%) adolescents aged 15 years, 82 (19%) adolescents aged 16 years, and 59 (13.7%) adolescents aged 17 years. Median heights in boys and girls increased by age and boys were significantly taller than girls (P<0.001). Median SBPs generally (not always) increased with age, but there was no significant difference between SBPs in boys and girls (P=0.212). However, DBP was significantly higher in girls than in boys (P=0.009) although the median were in the same range due to variations of number of subjects by age group. The SBPHR and DBPHR were also significantly higher in girls than in boys (P <0.001).

The SBPHR and DBPHR correlations to SBP, DBP, height, and age are shown in **Table 2**. Statistically significant correlations were found between SBPHR and SBP, height, and age (P<0.001). Likewise, DBPHR had significant correlations with DBP, height, and age (P<0.001 for DBP and height, P=0.008 for age). Strong, positive correlations were found between SBPHR and SBP (r = +0.876), as well as between DBPHR and DBP (r = +0.914). Weak, inverse correlations were found between SBPHR and

Table 1. The characteristics of study subjects based on age and sex

Age in years	Males Median (range)	Females Median (range)	P value*
Height, cm			
12 (n=28)	141.5 (133-151)	138.0 (99-151)	0.500
13 (n=88)	150.5 (127-161)	143.0 (99-155)	0.096
14 (n=79)	148.0 (127-161)	147.0 (133-160)	0.738
15 (n=96)	154.5 (136-168)	149.0 (135-161)	<0.001
16 (n=82)	158.5 (137-168)	150.0 (99-159)	<0.001
17 (n=59)	161.0 (150-172)	146.5 (129-161)	<0.001
Total (N=432)	151.0 (127-172)	146.0 (99-161)	<0.001
Systolic blood pressure, mmHg			
12 (n=28)	105.0 (85-130)	115.0 (90-130)	0.304
13 (n=88)	102.5 (80-130)	112.5 (85-140)	0.007
14 (n=79)	112.5 (90-130)	120.0 (90-135)	0.191
15 (n=96)	120.0 (90-135)	110.0 (85-130)	0.218
16 (n=82)	115.0 (90-155)	110.0 (80-140)	0.395
17 (n=59)	115.0 (100-135)	110.0 (85-130)	0.865
Total (N=432)	110.0 (80-155)	110.0 (80-140)	0.212
Diastolic blood pressure, mmHg			
2 (n=28)	60.0 (50-80)	75.0 (50-80)	0.043
13 (n=88)	62.5 (50-85)	70.0 (50-90)	0.005
14 (n=79)	65.0 (50-90)	75.0 (50-90)	0.045
15 (n=96)	70.0 (50-90)	70.0 (55-95)	0.710
16 (n=82)	70.0 (50-100)	70.0 (50-100)	0.826
17 (n=59)	70.0 (60-90)	70.0 (50-80)	0.516
Total (N=432)	70.0 (50-100)	70.0 (50-100)	0.009
Systolic blood pressure-to-height ratio, mmHg/cm			
12 (n=28)	0.753 (0.616-0.872)	0.849 (0.596-0.963)	0.079
13 (n=88)	0.746 (0.552-0.938)	0.815 (0.594-1.010)	0.015
14 (n=79)	0.751 (0.573-0.897)	0.816 (0.600-0.951)	0.109
15 (n=96)	0.752 (0.563-0.956)	0.738 (0.581-0.912)	0.971
16 (n=82)	0.728 (0.621-0.987)	0.731 (0.526-1.212)	0.601
17 (n=59)	0.697 (0.592-0.839)	0.788 (0.599-0.906)	0.009
Total (N=432)	0.741 (0.552-0.987)	0.769 (0.526-1.212)	<0.001
Diastolic blood pressure-to-height ratio, mmHg/cm			
12 (n=28)	0.449 (0.362-0.537)	0.549 (0.331-0.615)	0.007
13 (n=88)	0.464 (0.341-0.625)	0.519 (0.331-0.629)	0.011
14 (n=79)	0.444 (0.333-0.621)	0.510 (0.350-0.634)	0.024
15 (n=96)	0.479 (0.325-0.588)	0.467 (0.342-0.620)	0.522
16 (n=82)	0.449 (0.336-0.637)	0.469 (0.316-0.808)	0.233
17 (n=59)	0.429 (0.382-0.577)	0.486 (0.352-0.576)	0.005
Total (N=432)	0.446 (0.325-0.637)	0.489 (0.316-0.808)	<0.001

*Mann-Whitney test

Table 2. SBPHR and DBPHR correlations to SBP, DBP, height, and age

Variables		SBPHR	DBPHR
SBP	r (P)*	+0.876 (<0.001)	-
DBP	r (P)*	-	+0.914 (<0.001)
Height	r (P)*	-0.337 (<0.001)	-0.293 (<0.001)
Age	r (P)*	-0.170 (<0.001)	-0.128 (0.008)

*Spearman's correlation test

Table 3. The AUC of SBPHR and DBPHR for male and female adolescents

Variables	AUC	95%CI	P value
Males			
SBPHR	0.911	0.868 to 0.953	< 0.001
DBPHR	0.973	0.952 to 0.995	< 0.001
Females			
SBPHR	0.942	0.914 to 0.969	< 0.001
DBPHR	0.976	0.958 to 0.993	< 0.001

height, as well as DBPHR and height (r = -0.337 and r= -0.293, respectively). A very weak, inverse correlation was found between SBPHR and age, as well as DBPHR and age (r = -0.170 and r = -0.128, respectively). Hence, clinically significant correlations were not found between SBPHR or DBPHR and age or height, with r <0.4.

The ROC analysis resulted in accuracies of >90% for both SBPHR and DBPHR, in discriminating between hypertensive and non-hypertensive adolescents based on sex (P<0.001) (**Table 3**). (DBP $\geq$ 95th percentile) were 0.507 for boys and 0.541 for girls.

The diagnostic values of the SBPHR and DBPHR cut-off points for males and females are shown in **Table 5**. The sensitivities of SBPHR and DBPHR in boys and girls were > 93%. Specificities of SBPHR and DBPHR in boys and girls were > 80%. These values indicate that SBPHR and DBPHR have high sensitivities and specificities for detecting hypertension in adolescents. The positive predictive values of SBPHR and DBPHR in boys and girls were each <56%. However, as these tests are for screening purposes, some false positive results may be tolerated. The negative predictive values of SBPHR and DBPHR in boys and girls were very high, >97%, indicating that SBPHR and DBPHR may serve very well in distinguishing adolescents without hypertension.

Table 4. Various cut-off points of SBPHR and DBPHR for male and female adolescents

Variables	Cut-off points	Sensitivity (%)	Specificity (%)
SBPHR			
Males	0.782	100	79.7
	0.787	100	81
	0.794	100	82.4
Females	0.830	96	79.5
	0.836	94	82
	0.839	88	82
DBPHR			
Males	0.506	100	79.1
	0.507	100	81
	0.508	100	83.5
Females	0.540	100	78.6
	0.541	100	81.2
	0.543	100	81.6

Discussion

Our study subjects were adolescents aged 12 to 17 years in Singkuang, Mandailing Natal District of North Sumatera. Subjects' heights increased with age, with boys beyond 14 years old significantly taller than girls of the corresponding ages (P <0.001), similar to findings in a previous study.[1]

Biological sex affects blood pressure and arterial hemodynamics. Since females generally have smaller body size, their blood pressure is lower than that of boys.[14,18] We found no significant difference in SBP between boys and girls (P=0212), but DBP was significantly higher in girls than boys (P=0.009) although the median in the same range due to variations of number of subjects by age group. This discrepancy may be due to greater body mass index (BMI) in girls than boys, increasing with age, leading to higher DBP in girls than in boys.[1] Other studies also found that overweight/obese children had two to three times the risk of hypertension, than normoweight children.[19-21] However, we did not measure our subjects' weights, so their BMIs could not be determined. In addition to BMI, sex hormones are known to affect vascular function, influencing the production of endothelin-1 and nitric oxide, both of which affect contraction of the vascular endothelium. Decreased nitric oxide activity and increased endothelin-1 activity were found in adolescents with hypertension. Pubertal adolescents were prone to unstable nitric oxide levels and hypertension.[22]

Both SBPHR and DBPHR were significantly higher in girls than in boys (P<0.001), similar to previous studies.[1,2] Correlation analysis revealed an inverse relationship between SBPHR and DBPHR and

height, with r = -0.337 (P<0.001), indicating that the higher the ratio, the shorter the subjects. In our study, boys were significantly taller than girls (P <0.001), so the BPHR in boys was lower than that in girls.

We found significant correlations between SBPHR/DBPHR and SBP/DBP, height, and age (P<0.05). Both SBPHR and SBP as well as DBPHR and DBP had strong, positive correlations. However, inverse correlations were found between SBPHR/DBPHR and height, as well as SBPHR/DBPHR and age. The greater the height and age, the smaller the BPHR. These correlations were weak, with r <0.4. From a clinical standpoint, SBPHR and DBPHR are not influenced by age, so BPHR cut-off points were not determined by age. As such, the BPHR can be used in both tall and short adolescents. These results are consistent with previous studies which stated that BPHR was not associated with age and height.[1,2,23,24] Thus, BPHR is simpler to use than the gold standard NHBPEP tables.

In this study, we found that the accuracy of SBPHR and DBPHR for the diagnosis of hypertension in adolescents was >90% (P <0.001). As such, BPHR had a strong ability to discriminate between hypertension and non-hypertension in adolescent boys and girls. Previous studies also found the accuracy of BPHR to be >90% in adolescent populations in China, Nigeria, and the United States.[1,2,23]

We found the optimal cut-off points of SBPHR and DBPHR to be 0.787 and 0.507 in boys, respectively, and 0.836 and 0.541 in girls, respectively, with sensitivities >90% and specificities >80%. The gold standard requires the use of *Centers for Disease Control and Prevention* (CDC) weight-to-height percentile graphics[25] and the NHBPEP normative blood pressure tables.[3] Hence, the BPHR is simpler to use, as it requires remembering only four cut-off points. Thus, the difficulty in detecting hypertension in adolescents can be reduced.

Our BPHR cut-off points for defining hypertension were different from previous studies. Lu *et al.* studied in adolescents in China and found SBPHR and DBPHR cut-off points of 0.75 and 0.48 in boys, respectively, and 0.78 and 0.51 in girls, respectively.[2] Ejike reported SBPHR and DBPHR cut-off points of 0.75 and 0.51 in boys, respectively, and 0.77 and 0.50 in girls, respectively, in a study of Nigerian adolescents.[1] Also, Galescu et al. found SBPHR and DBPHR cut-off points 0.75 and 0.46 in boys, respectively, and 0.75 and 0.48 in girls, respectively.[23] In Indian adolescents, such cut-off points were 0.76 and 0.50 in boys, respectively, and 0.80 and 0.52 in girls, respectively.[24] These variations in cut-off points may be due to racial differences. Our study subjects were all of Mongoloid race in Singkuang, North Sumatera. Although the Lu *et al.* study also had subjects of Mongoloid race, they had a much larger sample size (13,136 *vs.* 432 adolescents).[2] Moreover, socioeconomic and cultural differences could have affected the cut-off points. It is possible that in the less developed countries, the wealthy readily adopt unhealthy lifestyles, characterized by smoking, sedentarism, and diets high in energy and fats.[26]

The diagnostic value of SBPHR and DBPHR for discriminating hypertension in adolescents compared to the NHBPEP tables,[2] was very good, with sensitivities of >93% and specificities of >80% for SBPHR and DBPHR in boys and girls. Similarly, Lu *et al.* and Ejike reported sensitivity and specificity >90% using the BPHR method,[1,2] while Galescu *et al.* and Ahmed *et al.* reported sensitivity and specificity of >80%.[23,24] Hence, SBPHR and DBPHR had a high ability to detect hypertension and distinguish between hypertensive and non- hypertensive adolescents.

Our positive predictive values for SBPHR and DBPHR were 45.2% and 38.7% in boys, respectively, and 55.9% and 42.4% in girls, respectively. Albeit low, these values were consistent with results of a previous study that reported a PPV range from 28% to 60%.[24] The low PPVs indicate that a hypertension diagnosis using SBPHR and DBPHR should be confirmed by the gold standard. In contrast, the negative predictive values of SBPHR and DBPHR in boys and girls were very high (>97%), respectively, consistent with previous studies.[1,2,23,24] High NPVs for SBPHR and DBPHR indicate that the BPHR is very good in discriminating non-hypertensive adolescents. Hence, adolescents diagnosed as normotensive using SBPHR and DBPHR, are actually non-hypertensive and can be eliminated after screening, thereby, simplifying the steps in detecting hypertension in adolescents.

This study had several limitations. First, the small sample size led to differences in the cut-off points, even in subjects of the same race. Second, we examined the adolescent population of only the Batak Mandailing tribe, therefore, our findings cannot be necessarily be

generalized to the population of Indonesia. However, previous studies stated that height does not depend on ethnic differences,[2] so it is likely that our findings can be used in different ethnic groups, until such time as more data becomes available.

In conclusion, the blood pressure-to-height ratio is a simple and easy diagnostic tool with high sensitivity, specificity, and negative predictive value for diagnosing hypertension in adolescents. Clinically, the BPHR can be used to screen for specific hypertensive adolescents, and to distinguish them from non-hypertensive adolescents. Adolescents diagnosed with hypertension using the BPHR method should undergo confirmation by the NHBPEP tables. However, those diagnosed as non-hypertensive can be eliminated. This method may be applicable to other ethnic groups, but should be validated in those groups.

Conflict of Interest

None declared.

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Original Article

Effect of oral administration of probiotics on intestinal colonization with drug-resistant bacteria in preterm infants

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Abstract

Background *Oral administration of probiotics in newborn preterm infants has been shown to be helpful, especially in reducing the incidence of necrotizing enterocolitis and overall mortality rates.*

Objective *To evaluate the effect of probiotic supplementation on intestinal colonization by antibiotic-resistant microorganisms in preterm infants receiving antibiotics in a neonatal intensive care unit (NICU).*

Methods *The prospective, randomized trial was performed in preterm infants who were hospitalized in the NICU at Baskent University Ankara Hospital between January 2011 and February 2012. A total of 51 infants were enrolled and randomly assigned to one of two groups: Group 1 (n=27) received probiotic therapy and Group 2 (n=24) did not receive probiotics. The probiotic used was Lactobacillus reuteri (Biogaia® AB, Sweden). Subjects underwent weekly nasal swab and stool cultures for a maximum of 6 weeks, and at the time of discharge if this was prior to 6 weeks. All positive cultures were further tested for culture-specific identification and antibiotic suceptibility.*

Results *A total of 607 cultures were evaluated. Positive cultures were found in 37.9% from Group 1 and 35.2% from Group 2. Intestinal colonization by antibiotic-resistant bacteria did not significantly differ between groups (P>0.05).*

Conclusions Oral supplementation with probiotics do not prevent the intestinal colonization of antibiotic-resistant microorganisms in preterm NICU patients who received antibiotic treatment. **[Paediatr Indones. 2017;57:91-8. doi: http://dx.doi.org/10.14238/pi57.2.2017.91-8].**

Keywords: *oral administration of probiotics; intestinal colonization; drug-resistant bacteria; preterm infants*

Preterm newborn infants who require intensive care are at increased risk for nosocomial infections caused by antibiotic-resistant microorganisms. For this reason, in preterm infants who remain in the NICU longer than 48 hours, the prevalence of nosocomial infections ranges from 6% to 22%.[1-5] Several studies have shown that supplementation with probiotics can prevent colonization of the gut by pathogenic microorganisms in preterm newborns.[1,3,4] Probiotics can help regulate enteral feeding, reduce parenteral nutrition dependence, enforce the intestinal mucosal barrier against bacteria, and increase levels of beneficial bacteria in the gut.[2,5-7] At the same time, probiotic therapy is reported to reduce frequencies of sepsis and necrotizing enterocolitis in preterm newborn infants.[2,5,7-10] Normally, the uterus is a sterile environment. As such, the intestinal microbiota starts developing shortly after birth in preterm infants, and the initial source of these colonizing microorganisms is the mother's flora.[1,3,4,11] Development of intestinal microbiota in preterm infants may also be delayed because of the hospital environment that consists of invasive procedures, antibiotic regimens, and

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late enteral feeding in the NICU.[12-14] Treatment with antibiotics can adversely affect the density and diversity of microorganisms in the intestine of the newborn.[15] Various studies have demonstrated that antibiotic-resistant microorganisms colonize preterm newborn infants in the NICU.[1,3,4,16,17]

Our aim in this study was to evaluate the effect of oral probiotic administration on the colonization of the intestine by antibiotic-resistant microorganisms in preterm newborn infants receiving antibiotics in the NICU.

Methods

This prospective study was performed in preterm newborn infants who were hospitalized in the NICU at Baskent University Ankara Hospital between January 2011 and February 2012. The Baskent University Clinical Research Ethics Committee approved the study (project number: KA11/138), and subjects' parents provided informed consent. All infants enrolled were born at ≤36 weeks of gestational age and required antibiotic treatment and/or prophylaxis. Infants with congenital anomalies and those undergoing intestinal surgeries were excluded.

Patients were randomly assigned to two groups, according to the order of NICU admission. A total of 51 patients were enrolled: Group 1 (n=27) received probiotic therapy and Group 2 (n=24) did not receive probiotics. The probiotic used was Lactobacillus reuteri (*Biogaia® AB*, Sweden). Oral probiotics were started on the day of birth. Each newborn in Group 1 received the probiotic directly (not mixed with any other intake) as an oral daily dose (1x108 cfu/day given as 5 drops once daily) during their stay in the NICU.[2]

Nasal swab and stool cultures were collected from all infants. In each case, these specimens were collected immediately upon admission to the NICU (prior to starting antibiotic treatment), at least once weekly throughout the hospital stay, to a maximum of 6 weeks, and at discharge if this was prior to 6 weeks. Each sample was incubated at a microbiology laboratory within 30 minutes of collection (see detailed laboratory methods below).

Other cultures (i.e., cultures of throat swabs, deep tracheal aspirates, endotracheal tube aspirates, blood and urine) were routinely taken from the patients included in the study. The relation of these culture results to the use of probiotic was investigated.

The following data were recorded for each infant during their stay in the NICU: prenatal, natal, and postnatal characteristics, diagnoses, clinical characteristics, surgical therapy and other interventions, prognosis, and complications (such as vomiting, diarrhea, sepsis, etc.) of probiotic treatment (for Group 1).

Cultures were plated and incubated at the *Baskent University Clinical Microbiology and Microbiology Laboratory*, and were evaluated by experts in the *Department of Infectious Diseases*. Specimens were plated on sheep blood agar, chocolate agar, and eosin methylene blue agar. Culture-specific identification and antibiotic susceptibility testing were performed for all microorganisms that grew in culture. The criteria of the *Clinical and Laboratory Standards Institute* were used to assess the antibiotic susceptibility of each microorganism.The methods used were the disc diffusion susceptibility test (Kirby-Bauer method) and determination of minimal inhibitory concentration (MIC).[18,19] The microbes that were cultured included methicillin-resistant *Staphylococcus aureus, Staphylococcus epidermidis*, Enterococcus spp., Klebsiella spp., *Escherichia coli, Pseudomonas aeruginosa*, Enterobacter spp, Acinetobacter spp , Serratia spp, Citrobacter spp., Proteus spp., and Candida spp. The microorganisms detected were classified according to their resistance to antibiotics.[20]

Statistical analysis was performed using the Statistical Package for the Social Sciences (*SPSS version 17.0, SPSS Inc.*, Chicago, IL, USA). The Shapiro-Wilk test was used to assess whether variables were normally distributed. The Mann-Whitney U test was used to compare the group findings. Results for categorical variables were analyzed using the Chi-square test. Within-group comparisons were made to assess weekly increases in quantity of microorganisms isolated from stool and nasal cultures, respectively. These weekly statistical comparisons were made using the Cochran Q test and the Monte Carlo method.

Results

The demographic characteristics of the two groups are summarized in **Table 1**. The groups' clinical characteristics are presented in **Table 2**. In total, 607 cultures were evaluated, with 351 from Group 1 and 256 from Group 2. One hundred thirty-three (37.9%) of the 351 Group 1 cultures were positive. Of the 133 isolates, 79 were Gram-negative microorganisms, 49 were Gram-positive, and 5 were fungi (only blood and catheter cultures). Ninety (35.2%) of the 256 Group 2 cultures were positive. Of the 90 isolates, 53 were Gram-negative and 37 were Gram-positive.

Table 3 shows other cultures were routinely taken from the patients included in the study. However, these culture results were not associated with oral administration of probiotics in both groups. **Table 4** shows the quantities of identified isolates cultured from each group's stool cultures at baseline (admission to NICU) and at weeks 1 through 6. **Table 5** shows the corresponding results for nasal swab cultures. The within-group comparisons of weekly numbers of isolates revealed weekly increases in quantity of microorganisms isolated from nasal cultures.

In this study most microorganisms were antibiotic-resistant ($P>0.05$). Very few of them were not antibiotic-resistant (**Table 6**).Colonization of intestine with antibiotic-resistant bacteria did not differ between the two groups ($P>0.05$). Klebsiella spp. isolated from a total of 26 cultures from both groups were positive for extended-spectrum beta-lactamase (ESBL), but *E. coli* isolated from 5 cultures were positive for ESBL from Group 1.

None of the infants in Group 1 developed side effects associated with the use of probiotics, such as diarrhea or vomiting.

Discussion

In this prospective, randomized trial, 607 cultures were evaluated in 51 preterm infants who received antibiotics in the NICU. Group 1 had significantly more antibiotic resistant microorganisms cultured from stool specimens, than Group 2. In Group1, the most common microorganisms isolated from all nasal swab cultures were Staphylococcus 20.0%, while in Group 2, the most common microorganisms isolated from all nasal swab cultures were Staphylococcus 17.6%.

The intestinal microbiota differs between term and preterm infants. Because preterm infants have immature host defenses, require invasive interventions such as central venous catheter or endotracheal tube insertion, and often have longer antibiotic treatment, they are at high risk for nosocomial and antibiotic-resistant infections. At the same time, colonization by bifidobacteria is delayed in the preterm infants.[15] A previous randomized clinical trial evaluated the effect of *Bifidobacterium lactis Bb12* supplementation on modifying gut microbiota in 69 preterm infants and found that supplementation with *B. lactis Bb12* did not reduce the colonization of antibiotic-resistant organisms.[15]

Other reports suggested that probiotics reduce intestinal inflammation and prevent colonization by pathogenic microorganisms in the gut.[2,3,21] Ren *et al.* reported that the intestinal bacterial colonization rate was lower in the group given probiotics than in the group without probiotics. In their study, *Klebsiella pneumoniae, E.coli*, and *Enterococcus faecium* were found in stool specimens of both the intervention and control groups.[22] In our study, we found that the use of probiotics in preterm infants did not prevent the development of antibiotic-resistant microorganisms. The differences between studies might be due to the diversity of invasive procedures, antibiotic regimens, and other treatments in the NICU. Ren *et al.* also reported that probiotics reduced the risk of sepsis in

Table 1. Demographic characteristics of the study subjects

Characteristics	Group 1 (probiotics) (n=27)	Group 2 (no probiotics) (n=24)	P value
Gender,* n			
Female	15	7	
Male	12	17	
Mode of delivery,** n			
Vaginal	3	2	0.058
Cesarean	24	22	0.739
Early membrane rupture,** n	7	5	
Mode of feeding, n			
Breastfed	22	20	0.699
Formula fed	5	4	0.830

*Chi-square test, **Mann-Whitney U test

Table 2. Clinical features, diagnoses, and interventions in the two study groups

Variables	Group 1 (n=27)	Group 2 (n=24)	P value
Gestational age, weeks			
Mean (SD)	32.5 (0.44)	33.1 (0.40)	0.312
Median (range)	32.7 (27-36)	33.4 (27-35)	
Birth weight, g			
Mean (SD)	1909.6 (111.75)	2048.7 (76.12)	0.242
Median (range)	1870 (840-2880)	2137 (930-2540)	
Apgar 1 minute			0.610
Mean (SD)	6.9 (0.21)	7.0 (0.24)	
Median (range	7.1 (5-9)	7.2 (3-8)	
Apgar 5 minutes			0.254
Mean (SD)	8.1 (0.17)	8.2 (0.20)	
Median (range	8.1 (6-10)	8.4 (5-9)	
Intubated, n	15	13	0.921
Ventilatory support, n	16	14	0.993
Umbilical venous catheter, n	21	14	0.135
Peripheral central catheter, n	3	4	0.565
Surfactant treatment, n	14	12	0.895
Use of antacid, n	8	9	0.552
Respiratory distress syndrome, n	14	12	0.895
Necrotizing enterocolitis, n	5	2	0.291
Sepsis, n	9	4	0.321
Patent ductus arteriosus, n	3	3	0.878
Bronchopulmonary dysplasia, n	3	1	0.357
Intubation duration, days			0.720
Mean (SD)	2.40 (0.84)	1.37 (0.35)	
Median (range	1 (0-19)	1 (0-6)	
Duration of umbilical venous catheter placement, days			0.105
Mean (SD)	6.6 (1.09)	4.0 (0.90)	
Median (range	6 (0-20)	2.5 (0-130	
Duration of peripheral central venous catheter placement, days			0.705
Mean (SD)	2.0 (1.18)	1.6 (0.78)	
Median (range)	0 (0-25)	0 (0-11)	
Duration of nasogastric tube placement, days			0.455
Mean (SD)	15.2 (3.42)	10.3 (1.98)	
Median (range	8 (1-70)	7.5 (1-37)	
Duration of total parenteral nutrition, days			0.638
Mean (SD)	6.9 (1.98)	4.3 (0.98)	
Median (range	5 (0-48)	7.5 (1-37)	
Duration of full enteral feeding, days			0.192
Mean (SD)	13.4 (2.21)	9.6 (1.26)	
Median (range	11 (3-60)	7.5 (0-25)	
Exposure to oxygen, days			0.549
Mean (SD)	9.7 (3.61)	6.3 (1.56)	
Median (range	2 (1-74)	2.5 (1-28)	
Time to first positive culture, days			0.237
Mean (SD)	7.1 (0.67)	6.0 (0.60)	
Median (range	7 (2-17)	5 (2-16)	
Total duration of antibiotic use, days			0.236
Mean (SD)	13.7 (2.35)	9.7 (1.31)	
Median (range	9 (6-45)	8 (3-30)	

Table 2. Clinical features, diagnoses, and interventions in the two study groups (continued)

Variables	Group 1 (n=27)	Group 2 (n=24)	P value
Hospital stay, days			
Mean (SD)	22.1 (3.52)	15.0 (1.82)	0.121
Median (range	15 (6.-74)	13 (6-43)	
Weight at discharge, g			0.278
Mean (SD)	2085.9	2148.3 (57.93)	
Median (range)	2050 (1620-2900)	2150 (1740-2700)	
Deaths, n	0	1	0.284

Table 3. Diagnostic value of IT ratio and procalcitonin as compared to blood cultures

	Culture type						
	Throat swab	DTA	ETA	Central catheter tip	Blood	Urine	Total
Group 1 (probiotics)							
Negative cultures	7	13	3	17	53	11	104
Enterococcus spp	0	0	0	1	0	2	3
Staphylococcus epidermidis	0	0	0	0	1	1	2
Stenotrophomonas maltophilia	0	3	1	0	0	0	4
E. coli	0	1	0	0	0	2	3
Klebsiella pneumoniae	0	0	0	0	0	2	2
Burgholderia spp	0	0	0	0	1	0	1
Candida parapsilosis	0	0	0	1	3	0	4
Group 2 (no probiotics)							
Negative cultures	7	11	2	8	41	7	76
S. epidermidis	0	0	1	1	0	0	2
Serratia marcescens	0	0	0	0	0	1	1
Streptococcus spp.	0	0	0	0	1	1	2
Total	14	28	7	28	100	27	204

DTA=deep tracheal aspirate; ETA=endotracheal tube aspirate

the preterm newborn infants.[22] In contrast, we found that intestinal bacterial colonization was higher in Group 1 than in Group 2, but the risk of sepsis did not increase in either group (**Table 4** and **5**).

Another study on very low-birth weight infants (VLBW, <1500 g), reported that colonization in stool samples were Lactobacillus sp. 71% and Klebsiella sp. 0%, within the first week of life without oral administration of probiotics.[23] Jacquot *et al.* reported that the most common bacteria in stool specimens found at 3 - 4 weeks postnatally was Clostridium.[24] The same study showed that Enterobacteriaceae accounted for less than < 10% and 44.4% in stool cultures at 6 and 8 weeks of life, respectively, and Bifidobacterium was < 10% at 8 weeks.[24] Rougé *et al.* investigated intestinal microbiota in 10 preterm infants. They reported that *Lactobacillus rhamnosus* and *Bifidobacterium longum* were in the intestinal flora of preterm infants who received probiotics. However, in preterm infants not receiving probiotics, Staphylococci was the first isolated bacteria in the intestinal flora.[25] Unlike these three reports, our study revealed higher Klebsiella spp in stool cultures in three weeks in both intervention and control groups. These differences may be explained by the blockage of saprophytic flora formation due to the use of antibiotics in both of our study groups. So, oral probiotic administration did not enhance the development of saprophytic flora. Vidal *et al.* investigated the impact of probiotics on the intestinal colonization of vancomycin-resistant enterococci (VRE) in mice receiving oral vancomycin. Administration of probiotics did not affect the density

Table 4. Microorganisms isolated from the weekly stool cultures for the two groups

	Admission to NICU	1 wk	2 wks	3 wks	4 wks	5 wks	6 wks
Group 1* (probiotics)							
Negative cultures	24	10	1	0	1	0	0
Klebsiella spp	1	8	13	5	3	0	0
Enterococcus	0	1	5	1	1	1	2
E. coli	1	3	4	2	0	1	0
Enterobacter	0	1	4	4	1	0	0
Staphylococcus epidermidis	1	3	1	2	1	0	0
Proteus spp	0	0	1	1	1	0	1
Stenotrophomonas maltophilia	0	1	1	1	0	0	0
Acinetobacter baumannii	0	1	0	1	0	0	0
Serratia marcescens	0	1	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0	1	0
Citrobacter spp	0	1	0	0	0	0	0
Total cultures**	27	30	30	17	8	3	3
Microorganism isolated, n(%)	3 (11.1)	20 (66.6	29 (96.6)	17 (100)	7 (87.5)	3 (1001)	3 (100)
Group 2* (no probiotics)							
Negative cultures	22	7	0	0	1	0	0
Klebsiella spp	0	6	13	8	1	1	1
Enterococcus spp	0	2	2	2	0	1	1
S. epidermidis	2	4	0	1	0	0	0
E. coli	0	0	2	1	1	0	1
Enterobacter spp	0	4	0	0	0	0	0
Proteus spp	0	1	1	1	0	0	0
Serratia SPP	0	0	1	1	0	0	0
A. baumannii	0	1	0	0	0	0	0
Total cultures**	24	25	19	14	3	2	3
Microorganisms isolated. n(%)	2 (8.3)	18 (72.0)	19 (100)	14 (100)	2 (66.7)	2 (100)	3 (100)

Notes: *Between the two groups weekly positive culture status in stool cultures was found significant by Cochran Q test (P=0.009). Positive culturing rates were higher in the probiotic group than in the no probiotic group. **Total cultures performed for the group. All patients had samples cultured on admission to the NICU. In following weeks, the numbers of cultures dropped as patients were discharged from hospital.

of VRE colonization in the gut.[26] Similarly, we found that the use of probiotics did not prevent development of resistant microorganisms in preterm infants.

The limitations of our study were the small sample size and the lack of ability to culture anaerobic microorganisms. In our study, rates of antibiotic-resistant microorganisms were found to be high in both groups. Clearly, our study shows that the use of probiotics does not prevent the colonization of antibiotic-resistant pathogens. We suggest that the antibiotic regimens and NICU conditions play the greatest role in the development of the intestinal microbiota and microbes cultured.

In conclusion, our study revealed that the use of probiotics do not prevent development of antibiotic resistant microorganisms in preterm infants receiving antibiotics in the NICU. Further studies may investigate the potential of oral supplementation of other probiotic strains in preventing antibiotic-resistant bacteria.

Conflict of Interest

None declared.

Table 5. Microorganisms isolated from the weekly nasal swab cultures for the two groups

	Admission to NICU	1 wk	2 wks	3 wks	4 wks	5 wks	6 wks
Group 1* (probiotics)							
Negative cultures	26	20	15	8	5	1	3
Staphylococcus epidermidis	0	6	8	5	1	2	0
Streptococcus pneumonia	0	1	1	0	0	0	0
Klebsiella spp	0	0	2	1	1	0	0
Enterococcus spp	2	0	0	0	0	0	0
E. coli	0	0	0	1	0	0	0
Serratia marcescens	0	0	1	0	0	0	0
Total cultures**	28	27	27	15	7	3	3
Microorganism isolated, n(%)	2 (7.1)	7 (25.9)	12 (44.4)	7 (46.6)	2 (28.5)	2 (66.6)	0
Group 2* (no probiotics)							
Negative cultures	24	17	12	5	2	0	0
S. epidermidis	0	4	4	5	0	2	0
S. pneumoniae	0	0	0	0	0	0	0
Enterobacter cloacae	0	0	1	0	0	0	0
Staphylococcus aureus	0	0	0	1	0	0	0
Klebsiella pneumoniae	0	0	0	1	0	0	0
Enterococcus faecalis	0	1	0	0	0	0	0
Stenotrophomonas maltophilia	0	0	1	0	0	0	0
S. marcescens	0	0	0	1	0	0	0
Acinobacter baumannii	0	0	0	0	0	0	1
P. aeruginosa	0	0	0	0	1	0	0
Total cultures**	24	24	18	13	3	2	1
Microorganisms isolated. n(%)	0	7 (29.1)	6 (33.3)	8 (61.5)	1 (33.3)	2 (100)	1 (100)

Notes: *Weekly positive culture status in nasal swab cultures was not significantly different between the two groups by Cochran Q test (P=0.097). **Total number of cultures performed for the group. In following weeks, the numbers of cultures dropped as patients were discharged from hospital.

Table 6. Distribution of microorganisms isolated from the two groups listed according to categories of antibiotic resistance

Category of antibiotic resistance	Group 1	Group 2	P value*
Not resistant	8 (6.5)	8 (8.5)	> 0.05
Resistant to one drug	13 (10.5)	7 (7.4)	> 0.05
Resistant to two drugs	5 (4.0)	6 (6.3)	> 0.05
Resistant to three or more drugs	105 (85.3)	73 (77.6)	> 0.05
Total	123 (100)	94 (100)	> 0.05

*Chi-square test

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Original Article

Duration of watching TV and child language development in young children

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Abstract

Background *Many factors contribute to language development in children. About 5-8% of children in Indonesia experience delayed language skills. Young children need appropriate stimulation for optimal development. Children who watch television (TV) for long periods of time may receive less two-way interaction, the appropriate stimulation for learning. As such, shorter duration of the appropriate stimulation may impede language development in small children.*

Objective *To assess for an association between duration of watching TV and language development in young children.*

Methods *This cross-sectional study was done with primary data collected from questionnaires. Subjects, aged 18 months to 3 years, were from a Jakarta-area community health center (Puskesmas) Jatinegara and the Pediatric Growth and Development Clinic, Cipto Mangunkusumo Hospital, Jakarta. Their language development was tested using the Developmental Pre-screening Questionnaire (Kuesioner Pra Skrining Perkembangan, KPSP) and the Early Language Milestone (ELM Scale 2) test.*

Results *From a total of 84 subjects, 47 (56%) had normal and 37 (44%) had delayed language development. Duration of watching TV was categorized as <4 hours per day or >4 hours per day. Children who watched TV >4 hours/day (OR 4.4; 95%CI 1.68 to 11.7; P=0.002), and children who watched both Indonesian and English language TV programs (OR 14.7; 95%CI 1.77 to 123.0; P=0.004) had higher risk of language delay. Other variables such as sex, first age exposed to TV, use of gadgets, and TV in the bedroom had no significant associations with delayed language development.*

Conclusion *Children who watch TV >4 hours/day have four times higher risk of developing language delay. In addition, those who watch TV programs in both Indonesian and English, also have a 14.7 higher risk of delayed language development.* **[Paediatr Indones. 2017;57:99-103. doi: http://dx.doi.org/10.14238/pi57.2.2017.99-103].**

Keywords: *child TV viewing; duration of watching TV; delayed language development*

Proper stimuli are needed for optimal child development. Parents should encourage their children to be physically active, in order to develop their potential.[1] However, children also like to watch TV, as many programs are offered for adults as well as children as target audiences. Some parents find TV to be helpful, especially while attending to household tasks, because their children are entertained by TV programs. However, some parents may not realize that despite the calming influence of TV on children, frequent TV viewing is a bad habit for children, as it may impact their language development.[2]

The American Academy of Pediatrics (AAP) recommends that children >2 years of age watch TV or use other media (gadgets) for no longer than 2 hours per day. In addition, children under 2 years, should not be exposed to media at all.[2] The time spent on TV or other media can inhibit child language development. However, TV programs and technological gadgets are interesting and always being improved. Their developers persuade parents to provide them as educational tools for children. Duch *et al.*[3] reported that 82% of children aged one year

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watched TV everyday; some children aged 2 years watched TV longer than two hours per day. They also found that as children grew older they tended to watch more TV. This habit should be changed in order to achieve better development, as not only language development is affected, but cognitive development and academic achievement may also be affected as the child gets older.[3,4]

The Indonesian Pediatric Society (Ikatan Dokter Anak Indonesia) stated that 5-8% of preschool children have speech and language delays.[5] According to Suwarba et al. speech and walking delays are the most common developmental problems in children seen at Cipto Mangunkusumo Hospital.[6] Many etiologies can influence language development, one of which might be related to family habits, including time spent in front of the TV. Almost all Indonesian families have a television in the home, many of whom have a television in the bedroom.

Past studies on TV exposure in children and how it affects child language development have been inconclusive. Ruangdaraganon et al. found no association between watching TV and language development in 2-year-olds in Thailand.[7] However, Chonchaiya *et al.* found that 56 out of 110 children had delayed language development due to TV-watching habits. They also found that children younger than one year who watched TV more than two hours daily were at much higher risk of delayed language development.[8]

We aimed to assess for an association between duration of watching TV and child language development in toddlers. We chose an age range of 18 months to 3 years because language development, as well as brain development, occurs most rapidly in children below 5 years of age.

Methods

We performed a cross-sectional study with simple random sampling for subjects aged 18 months to 3 years. The study was conducted at the Pediatric Growth and Development Clinic, Department of Child Health, Cipto Mangunkusumo Hospital and community health center (Puskesmas) Jatinegara, Jakarta from January to May 2015. A total of 84 children were included in this study. Language development was measured with the Developmental Pre-screening Questionnaire (*Kuesioner Pra Skrinning Perkembangan or KPSP*) which had been used widely in Indonesia, and the *Early Language Milestone* (ELM Scale – 2) test.[9,10]

The exclusion criteria was children with genetic disease. Children who failed to complete the KPSP and ELM Scale-2 test as well as children whom parents did not complete the questionnaire were also excluded. Children with KPSP scores of 9-10 were considered to be normal in all aspects of development, including language development. However, children with KPSP scores of 8 or below underwent further testing with ELM Scale-2 to determine the status of their language development. The ELM Scale-2 was used to assess the language development in subjects, which consisted of three divisions namely auditory expressive, auditory receptive, and visual. Assessment was done according to the age of the subject. Subject were classified to have normal language development if she/he passed all the three divisions, and categorized to have delayed language development if subject failed on one of three divisions. Based on the results of the KPSP and ELM Scale-2 test, children were categorized as having delayed or normal language development. Parents filled questionnaires on their children's TV habits. Watching TV was assumed as the time when the children were put in front of TV, whether with parents or alone. The durations of watching TV were grouped into <4 hours or >4 hours per day. Types of language used in TV program were categorized into Indonesian or English language, or both. Assessment of duration of gadget usage per day was based on the question "How many hours does the child spent on playing gadget per day?" filled by the parents in the questionnaire. The term gadget includes tablets, computer, and laptop. The descriptive data was analyzed by Chi-square test using *SPSS version 17*. Results with P values <0.05 were considered to be statistically significant.

Results

Of 84 children, those with normal language development (47 subjects) consisted of 57% females and 43% males. Children with delayed language development (37 subjects) consisted of 41% females

and 59% males. We found that 95% of children with delayed language development and 94% of children with normal language development had been first exposed to TV at less than 2 years of age. Regarding the duration of watching TV, children who watched TV for more than 4 hours/day had a higher risk of delayed language development (OR 4.4; 95%CI 1.68 to 11.7; P=0.002).

Other variables, such as sex, age that the child was first exposed to TV, the use of gadgets, and TV in the bedroom, were also evaluated, as shown in **Table 1**. There were 57% of children with normal and 62% of children with delayed language development who used gadgets (P=0.823). In addition, 49% of subjects with normal language development had a TV in their bedroom, while 70% of those with delayed language had a TV in the bedroom, but this difference was not significant (P=0.74). About 98% children with normal language development watched Indonesian TV programs, and only 2% watched both Indonesian and English TV programs. In children with delayed language development, 76% watched Indonesian TV programs and 24% watched Indonesian and English TV programs. Hence, significantly more subjects with normal language development watched only Indonesian programs, than did subjects with delayed language development (OR 14.7; 95%CI 1.77 to 123.02; P=0.004).

We found that the mean age of children were 26.1 months in the normal language development group, with the youngest being 10 months and the oldest being 36 months, as shown in **Table 2**. The mean age in the delayed language development group was 29.3 with the youngest being 18 months and the oldest being 36 months. Mean daily TV times was 2.9 hours in the normal language development group, with the shortest duration is less than 1 hour and the longest duration is 10 hours per day. For the delayed language development group the mean daily TV times was 4.4 hours, with the shortest duration is less than 1 hour and the longest duration is 12 hours per day. The mean daily gadget times were 1.5 hours for those with normal language development and 1 hour for those with delayed language development. The shortest duration is 30 minutes and the longest duration is 2 hours per day both in the normal and delayed language development group.

Discussion

Many factors contribute to child language development. Development occurs progressively and varies at different ages. Children need appropriate stimulation for optimal development.[11,12] Children imitate the speech of the people around them, often

Table 1. Associations between language development in children aged 18-36 months and variables

Variables	Language development		Odds ratio (95%CI)	P value
	Normal (n=47)	Delayed (n=37)		
Gender, n(%)				
Male	20 (43)	22 (59)	0.5 (0.21 to 1.21)	0.187
Female	27 (57)	15 (41)		
Daily TV exposure, n(%)			4.4 (1.68 to 11.7)	0.002
≤ 4 hours	38 (81)	18 (49)		
> 4 hours	9 (19)	19 (51)		
First exposed to TV, n(%)			0.8 (0.13 to 5.29)	1.000
< 2 years old	44 (94)	35 (95)		
≥ 2 years old	3 (6)	2 (5)		
Use of gadgets, n(%)			0.8 (3.41 to 1.98)	0.823
Yes	27 (57)	23 (62)		
No	20 (43)	14 (38)		
TV in bedroom, n(%)			0.4 (0.16 to 1.0)	0.74
Yes	23 (40)	26 (70)		
No	24 (51)	11 (30)		
TV language, n(%)			14.7 (1.77 to 123.02)	0.004
Indonesian	46 (98)	28 (76)		
Indonesian & English	1 (2)	9 (240		

Table 2. Mean age, duration of watching TV, and gadget use in children with normal and delayed language development

Variables	Meang age (SD), months	Mean daily TV time (SD), hours	P value	Mean daily gadget time (SD), hours	P value
Normal language development	26.1 (6.9)	2.9 (1.8)	0.025	1.5 (0.7)	0.067
Delayed language development	29.3 (7.7)	4.4 (3.2)		1 (0.9)	

mispronouncing words; for example, instead of saying, "hand," they say, "and." Eventually they will learn to pronounce words correctly, through stimulation and practice.[13,14] There are many ways to provide stimulation so that children improve their language development. Some parents believe that watching TV is one such method,[7] while TV may actually lead to language delays. Preventing children from watching TV is not easy, because TV is an interesting source of entertainment media at home. Some factors that influence watching TV are behavioral, such as total hours of daily sleeping, environmental factors such as family habit of watching TV, having TV in the bedroom, and non-parental child care. A child's biological and demographic factors include sex, age, ethnicity, and firstborn status; while the family's biological or demographic factors include parental education, income, and age.[3]

The AAP recommends that children watch TV only after the age of 2 years, and not exceeding 2 hours per day.[1] We found that 79 of 84 subjects started watching TV before 2 years of age. This observation suggests many parents are unaware of the potentially negative effects of watching TV at an early age. Of these 79 subjects, 44 had normal and 35 had delayed language development. Only 5 of our subjects began watching TV at ≥2 years of age.

A previous study showed a significant association between male sex and delayed language development.[7] In our study, the percentage of males with delayed language development was higher (59.5%) than for females, but the difference was not significant. We noted two significant variables related to delayed language development. First, children who had a >4 hour/day duration of watching TV had 4 times the risk of delayed language development (OR 4.4; 95%CI 1.68 to 11.7; P=0.002). Similarly, Chonchaiya *et al.* found that children who were first exposed to TV at less than 12 months of age and spent more than 2 hours daily watching TV had six times the risk delayed language development.[8] In our subjects with normal language development, 81% watched TV <4 hours daily. However, their mean duration of watching TV (2.9 hours daily) still exceeded the AAP recommendation (<2 hours). Subjects with delayed language development watched TV for a mean 4.4 hours daily.

The second significant variable related to developmental delays in language was the viewing of TV programs in two languages, Indonesian and English (OR 14.7; 95%CI 1.77 to 123.02; P=0.004). Children who watched TV programs in Indonesian and another language, most likely English (with Indonesian as the main language used at home), had 14.7 times the risk of delayed language development. The use of a language which is not often heard in daily life may confuse them. When children watch TV in a language different from their primary language, without their parents or caregivers to interact and guide them, they will likely have difficulty understanding the TV program. Learning is about repetitively receiving a stimulus, step-by-step.[11] Young children easily understand words they often hear due to direct interaction with the speaker. However, hearing words in another language on TV, is passive learning from TV. Furthermore, learning two languages at once may confuse the child and inhibit optimal learning, as children differ in their ability to learn. Moreover, differences in grammatical order (of Indonesian and English language) may confuse children in constructing sentences and may cause blended use of language.[13,14]

Although language development is complex, stimulation is one of the most important factors. Learning occurs in response to stimuli.[2] As such, watching TV itself may not directly affect language development, but the lack of stimuli does. In fact, the dangerous aspect of children watching TV is using the TV to replace interaction with parents or caregivers. As such, children spending long periods of time in

front of the TV, rather than interaction with parents, may affect their language development.[15,16]

In our study, having a TV in the bedroom was not significantly associated with language development, however we found more than half of children with delayed language development (70.3%) had a TV in their room. Besides TV, other entertainment media at home in the form of gadgets were not associated with child language development in our study. However, the use of gadgets should be evaluated in more detail, as to the suitability of various types of gadgets and software applications.

In conclusion, children who watch TV >4 hours/day have four times higher risk of developing language delay. In addition, those who watch TV programs in both Indonesian and English, also have a 14.7 higher risk of delayed language development.

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Conflict of Interest

None declared.

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Original Article

Duration and dose of antiepileptic drugs and serum calcium levels in children

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Abstract

Background Many factors contribute to language development in chilBackground Antiepileptic drugs (AEDs) may affect calcium metabolism through several mechanisms. Much evidence has confirmed that carbamazepine and valproic acid, as the most widely used AEDs in epileptic children, leads to decreased serum calcium levels. This effect was suggested to be time and dose dependent. However, correlations between AEDs and calcium levels in Indonesian epileptic children have not been well studied.

Objective To investigate possible correlations between total calcium levels and durations of therapy as well as doses of carbamazepine and valproic acid.

Methods This analytical, cross-sectional study was performed from March to May 2015 in the Neuropediatric Outpatient Ward of Mohammad Hoesin Hospital, Palembang, South Sumatera. A total of 60 epileptic children taking carbamazepine and or valproic acid monotherapy were included and grouped accordingly. A single blood test was done for every participant to measure total serum calcium level. Correlation between daily dose or duration of AED with calcium level was assess using the Spearman-rho test.

Results The mean total serum calcium levels in the carbamazepine and valproic acid groups were 9.48 (SD 0.83) mg/dL and 9.58 (SD 0.63) mg/dL, respectively. There was a statistically significant moderate correlation between the duration of carbamazepine therapy and total calcium level ($r = 0.36$; $P=0.001$). The cut-off point for duration of therapy was 23 months. There were no significant correlations between total calcium level and mean daily carbamazepine dose, nor between total calcium level and duration and dose of valproic acid therapy.

Conclusion Longer duration of carbamazepine therapy is associated with low total serum calcium level, but carbamazepine dose is not. In addition, duration and dose of valproic acid are not associated with low total serum calcium level. **[Paediatr Indones. 2017;57:104-7. doi: http://dx.doi.org/10.14238/pi57.2.2017.104-7]**.

Keywords: *calcium level; therapeutic duration; drug dose; antiepileptic drugs*

Antiepileptic drugs (AEDs) are the primary choice of therapy for epileptic patients at any age and gender. Treatment is sometimes prolonged and may require large doses as well as drug combinations. Therefore, the adverse effects of AEDs should be considered during treatment. One such effect is on calcium metabolism.[1,2] Calcium plays an important role in various physiological functions in the body including the blood clotting process, sodium and potassium cell membrane potential maintainance, signal transduction between hormone receptors, neuromuscular excitability, integrity of cell membrane, enzymatic reactions, neurotransmission, and bone structure formation. Clinical symptoms of hypocalcemia range from mild to severe. In plasma with calcium concentration 50% below normal, peripheral nerve fibers become excited, spontaneously spreading impulses to the peripheral skeletal muscle and triggering tetanic muscle contractions or even seizures. Extreme hypocalcemia can cause cardiac dilatation, blood clotting disorders, and death.[3]

Antiepileptic drugs have been widely reported

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to reduce serum calcium levels, which interferes with bone metabolism, decreasing bone density mainly through induction of the cytochrome P450 (CYP450) enzyme system in the liver. Decreased calcium levels elevate parathyroid hormone and increase bone turnover characterized by hyperphosphatemia. This mechanism mainly occurs in the group of enzyme-inducers, such as phenobarbital, phenytoin, and carbamazepine. However, recent research also showed that non-enzyme inducing AEDs, such as valproic acid, may also cause hypocalcemia through inhibitory effects of the AED metabolites on calcium ions.[1,2] These effects have been suggested to be time and dose dependent. Many studies reported that epileptic children treated with AEDs (carbamazepine or valproic acid) over a certain period of time experienced decreased total calcium levels.[4-6] Furthermore, dose reductions led to normalization of the calcium concentration.[7]

The effects of AED on total serum calcium levels in epileptic children in Indonesia have not been well studied. The objective of this study was to assess for possible correlations between durations of therapy and doses of AEDs (carbamazepine or valproic acid as the most widely used AEDs in epileptic children) and total serum calcium levels.

Methods

This cross sectional study was conducted in the Neuropediatric Outpatient Department of Mohammad Hoesin Hospital, Palembang, South Sumatera, from February to April 2015. We recruited subjects by consecutive sampling. Inclusion criteria were all epileptic patients aged between 6 months to <15 years, who had undergone valproic acid or carbamazepine treatment for at least 1 month, and whose parents provided informed consent. Exclusion criteria were children with pre-existing chronic kidney disease, liver failure, malnutrition, or hypoalbuminemia, as well as those who took other AEDs, calcium or vitamin D supplementation, or steroid or diuretic therapy.

Duration of therapy was defined to be the length of AED usage on a regular basis, expressed in months. Dose of therapy was defined to be the average maintenance AED dose in the past month, expressed in mg/kg/day. Total calcium level was defined to be blood calcium level obtained through venous blood test, including calcium ions, protein-bound calcium, and organic or complex–bound calcium. Low total calcium levels (hypocalcemia) was defined as a total calcium level below the reference value, according to age.[8]

We collected data on duration and dose of AEDs by history taking and retrieving data from the medical charts. Non fasting blood tests was done to measure total serum calcium level. Total calcium levels were measured by *COBASS INTEGRA®*.

Spearman's correlation test was used to analyze the correlation between the duration as well as the dose of valproic acid or carbamazepine and total calcium level. Significance was set at $P<0.05$ with 95% confidence intervals (CI).

Results

From February to April 2015, 30 epileptic children taking carbamazepine and 30 others taking valproic acid met the inclusion criteria. There were more males in the carbamazepine group and more females in the valproic acid group. The median ages in the carbamazepine and valproic acid groups were 52.5 and 57.5 months old, respectively. The median durations of therapy were 13 months in the carbamazepine group and 12 months in the valproic acid group. The median maintenance doses were 15 mg/kg/day in the carbamazepine group and 20 mg/kg/day in the valproic acid group. The mean total serum calcium levels were 9.48 mg/dL in the carbamazepine group and 9.58 mg/dL in valproic acid group. The characteristics of subjects are presented in **Table 1**.

Hypocalcemia was found in 13 patients (22% of all subjects), 9 patients (30%) in the carbamazepine group and 4 patients (13%) in the valproic acid group. The distribution of subjects' total calcium levels in both groups are shown in **Table 2**.

There was a statistically significant moderate correlation between the duration of carbamazepine therapy and low total serum calcium level, with a weak correlation coefficient ($r=0.36$; $P=0.001$). There was no correlation between daily carbamazepine dose with total calcium level ($r=0.03$; $P=0.878$).

For children taking valproic acid, there seems to be a weak correlation between valproic acid and total serum calcium although this was not statistically

Table 1. Characteristics of subjects (n=60)

Characteristics	Therapy group	
	Carbamazepine (n=30)	Valproic acid (n=30)
Gender, n		
Male	16	12
Female	14	18
Median age (range), months	53 (9-174)	58 (12-192)
Median duration of therapy (range), months	13 (2-72)	12 (2-108)
Median dose (range), mg/kg body weight/day	15 (5-30)	20 (10-40)
Mean total calcium level (SD), g/dL	9.48 (0.83)	9.58 (0.63)

Table 2. Distribution of hypocalcemia in the carbamazepine and valproic acid groups

Antiepileptic drugs	Calcium level		Total
	Low	Normal	
Carbamazepine, n	9	21	30
Valproic acid, n	4	26	30
Total	13	47	60

significant (r=0.35; P=0.057). No significant correlation between average daily dose of valproic acid and total serum calcium levels (r=0.11; P=0.56) was found.

Discussion

Our study demonstrated moderate correlations between total serum calcium level and carbamazepine duration of therapy. Valproic acid therapeutic duration also seemed to moderately correlate with total calcium level although this was not statistically significant. No correlations were found between daily dose of AED and total calcium level.

In the carbamazepine group, the mean total calcium level was within normal limits, and 9 subjects (30%) had total calcium levels below normal. Of the 9 subjects, the lowest calcium level was 8.1 mg/dL in 2 subjects but they had no symptoms of hypocalcemia. The best explanation of this effect was that carbamazepine activated the orphan nuclear receptor, pregnane X receptor (PXR), which shares 60% homology in their DNA-binding domains to the vitamin D receptor (VDR), and is expressed in the intestine, kidney, and liver. The PXR has been shown to mediate induction of CYP2 and CYP3, the cytochrome P450 enzymes involved in drug metabolism. Emerging evidence shows that these PXR activators can increase the expression of the CYP24, a VDR target gene, in cultured cells and in vivo in mice. CYP 24 is an enzyme that directs the side chain oxidation and cleavage of 25(OH)2 D3 and 1ß, 25 (OH)2D3 to carboxylic acid end products (calcitroic acid), resulting in lower cellular concentration of active vitamin D. This induces a state of vitamin D deficiency and results in hypocalcemia, secondary hyperparathyroidism, and increased bone turnover, predisposing to low bone density and bone loss. However, this does not explain hypocalcemia with valproic acid reported in some studies, as valproic acid is an inhibitor of cytochrome P450 enzymes and is not among the known activators of PXR.[9] In the valproic acid group, mean calcium levels were generally within normal limits, only 4 subjects (13%) had low calcium levels according to age, and no hypocalcemia symptoms were observed in these patients either. Other reports suggested that valproic acid might cause hypocalcemia through its inhibitory effects on calcium ions by its metabolites.[1,2,7]

Correlation analysis revealed a positive and significant correlation between duration of carbamazepine therapy and low calcium level, though the strength of correlation was weak. Median durations of therapy in carbamazepine group was found to be at 23 months. This median duration was much longer than that of a previous study (2009) that showed a significant decrease in calcium level after 60 days of therapy on a maintenance dose, and a further decrease after 90 days.[6] An Indian study conducted in 114 epileptic children treated with AEDs (carbamazepine, phenobarbital, phenytoin, valproic acid, and combinations) who initially had higher levels of calcium and vitamin D, also reported the decline of calcium and vitamin D levels after 6 months of AED therapy on maintenance doses. These contrasting results may be due to differences in methodology and/or study populations.

We found no significant correlation between carbamazepine dose and total calcium levels, in contrast to another study reporting a significant decline of vitamin D and calcium levels after 6 months carbamazepine therapy at maintenance doses.[10]

Also, no significant correlation was found between duration of valproic acid therapy and total calcium level, nor between dose of valproic acid and total calcium level.

In our subjects, hypocalcemia induced by AED was considered to be mild, with the largest decrease not exceeding 11% below normal calcium levels. As such, we would expect to not observe hypocalcemic symptoms. We also noted that hypocalcemia was more common in the carbamazepine group compared to the valproic acid group. Beyond the effect of elucidating the different mechanisms of hypocalcemia between drugs, this observation might provide the impetus for future research comparing AEDs' effects on calcium levels so that pediatricians can choose the appropriate AED to minimize the risk of hypocalcemia and prepare supplementation if necessary.

Some limitations of this study were that we examined total calcium levels rather than ionized calcium to represent the calcium levels because our facilities lacked the capability to examine ionized calcium levels. Another limitation is that the sample size is limited and that is why perhaps we did not pick up statistically significant correlation in the valproic acid group. Calcium ions actively play an important role in metabolism and are considered to represent 50% of total calcium.[11] The measurement of total calcium was considered sufficient to represent the state of calcium ions, especially in medical centers with limited facilities. However, the ionized calcium levels would have given better results in terms of the effects of valproic acid metabolites. Also, we did not examine the base profile levels of vitamin D and parathyroid hormone, which are known to have important roles in calcium metabolism.10 Decreased levels of calcium could possibly be due to the low levels of vitamin D or parathyroid hormone, or influenced by various factors such as sun exposure and hypoparathyroidism. Another limitation was the possibility that consumption of high calcium foods a few hours before the blood test affected total calcium levels.

In conclusion, moderate positive correlation is found between duration of carbamazepine therapy and total serum calcium. No correlation is found between daily dose of AED and total calcium level. Further study is recommended to compare AED side effects on calcium metabolism in a clinical trial.

Conflict of interest

None declared.

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Original Article

Screening for nutritional risk in hospitalized children: comparison of two instruments

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Abstract

Background Malnutrition in hospitalized children has negative impact on morbidity, mortality, length of stay, and health-care cost. A simple screening tool is needed to detect hospital malnutrition risk in children.

Objective To compare the level of agreement of the *Screening Tool for Malnutrition in Pediatrics* (STAMP) and *Pediatric Nutritional Risk Score* (PNRS) with anthropometric measurements, as screening tools for hospital malnutrition in children.

Methods A cross-sectional study was conducted from February to July 2014 in the Pediatric and Surgery Wards at H. Adam Malik Hospital, Medan, North Sumatera. Inclusion criteria were children aged 2 to 18 years who were hospitalized for more than 72 hours. Subjects were screened using STAMP and PNRS, and underwent anthropometric measurement on admission. The weight measurements were repeated on the 3rd and 7th days, and just before discharge. The STAMP and PNRS results were compared in terms of level of agreement with anthropometric measurements. Data were analyzed by Kappa value and Spearman's correlation test.

Results A total of 127 children were screened with both instruments. The PNRS had slight agreement with hospital malnutrition prevalence (K=0.175; P=0.028), while STAMP had not (K=0.080; P=0.193). Both screening tools had weak positive correlations with length of stay, but the correlation was stronger for PNRS than for STAMP (r=0.218; P=0.014 *vs.* r=0.188; P=0.034, respectively). The prevalence of hospital malnutrition was 40.9%.

Conclusion The PNRS screening tool has slight agreement with anthropometric measurement for identifying hospital malnutrition risk in children. **[Paediatr Indones. 2017;57:117-23; doi: http://dx.doi.org/10.14238/pi57.3.2017.117-23]**.

Keywords: *hospital malnutrition; STAMP; PNRS; anthropometric*

Hospital malnutrition (HM) is malnutrition that occurs in hospitalized patients.[1] Many factors contribute to the development of HM, such as decreased dietary intake caused by anorexia, feeding difficulties, side effects of medication, and other external factors such as invasive diagnostic or therapeutic procedures.[2] Poor nutritional status in hospitalized children has been associated with negative outcomes, including longer recovery time, greater requirement for intensive care, more complications, nosocomial infections, and even death.[3] The prevalence of HM varies depending on the criteria and parameters used to define malnutrition. In European countries and the United States during a ten-year period, 6.1 to 14% of hospitalized children were malnourished.[4] Two Indonesian studies reported higher prevalence of HM, from 24.3 to 24.8%.[5,6]

In order to prevent HM, it is important to promptly identify the nutritional risk in hospitalized children, using nutritional screening tools. Several instruments have been developed, but there is a paucity of study on the application of these tools. The

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Pediatric Nutritional Risk Score (PNRS) and *Screening Tool for the Assessment of Malnutrition in Pediatrics* (STAMP) have been developed and validated by some institutions.[7,8] We aimed to compare the level of agreement between PNRS and STAMP with anthropometric measurement, to identify hospital malnutrition risk in children.

Methods

A cross-sectional study was conducted from February to July 2014 in the Pediatric and Surgery Wards of Haji Adam Malik Hospital, Medan, North Sumatera. Both PNRS and STAMP scores were determined for all eligible pediatric patients. The inclusion criteria were children aged two to 18 years admitted to either the Pediatric or Surgery Ward, with length of stay at least 72 hours, who could undergo height measurement in a standing position, and whose parents gave informed consent. Children who were moved to the intensive care unit, had decreased consciousness or neurologic deficits such as spasticity, were diagnosed with congestive heart failure or nephrotic syndrome, were excluded. Further information was collected on age, sex, weight, height, length of stay, and reason for admission (disease category). We provided information about this study to the parents and subjects prior to data collection. This study was approved by the Health Research Ethics Committee of University Sumatera Utara.

Subjects' weights and heights were measured on the day of admission. Weight measurements were repeated on the 3rd and 7th days, and just before discharge, using a calibrated electronic scale (*Camry®*, China; precision 0.1 kg). Heights were measured using a calibrated 2-meter microtoise (precision 0.5 cm). Nutritional status was determined according to the *World Health Organization* (WHO) growth chart for children ≤ 5 years old,[9] and the *Centers for Disease Control* (CDC) growth chart for children aged > 5 years.[10] Malnutrition was defined based on Waterlow criteria, which classified subjects into normal, mild-moderate malnutrition, severe malnutrition, overweight, and obese.[11] Hospital malnutrition was diagnosed if there was weight loss ≥ 2% for length of stay ≤ 7 days, 5% for length of stay 8 – 30 days, or 10% for length of stay > 30 days.[7]

Application of screening tools to assess nutritional risk was performed within the first 48 hours of admission. The PNRS score consisted of three parameters, namely, disease pathology, pain, and food intake. Disease pathology was classified as mild (grade 1) for conditions involving mild stress factors, e.g., admission for diagnostic procedures, minor infections not necessarily requiring hospitalization, other episodic illnesses, or minor surgery. Grade 2 conditions involved moderate stress factors, e.g., severe but not life-threatening infection, routine surgery, fracture, chronic illness without acute deterioration, or inflammatory bowel disease. Grade 3 conditions involved severe stress factors, e.g., AIDS, malignancy, severe sepsis, major surgery, multiple injuries, acute deterioration of chronic disease, and major depression.[7] Pain was assessed using a visual analogue scale with rating from 0 (no pain) to 10 (worst pain imaginable). The cut-off point was a rating > 4.[12] Food intake was recorded by the investigator using 24-hour dietary recall. Score 0 was given for no pain, food intake > 50%, and grade 1 disease

Table 1. The pediatric nutritional risk score[7]

Risk factors [coefficients]		Score	Nutritional risk
Pathology of disease	Pain [1] and/or food intake < 50% [1]		
MIld (grade 1) [0]	Neither	0	Low
Mild (grade 1) [0]	One applies	1	Moderate
MIld (grade 1) [0]	Both apply	2	Moderate
Moderate (grade 2) [1]	Neither	1	Moderate
Moderate (grade 2) [1]	One applies	2	Moderate
Moderate (grade 2) [1]	Both apply	3	High
Severe (grade 3) [3]	Neither	3	High
Severe (grade 3) [3]	One applies	4	High
Severe (grade 3) [3]	Both apply	5	High

pathology. Score 1 was given for pain, food intake < 50%, and grade 2 disease pathology. Score 3 was given for grade 3 disease pathology. The total score of 3 parameters was recorded as the hospital malnutrition risk score, which ranged from 0-5. The PNRS score is described in **Table 1**.

The STAMP score assessed three elements: clinical diagnosis, nutritional intake, and anthropometric measures. Clinical diagnoses were divided into definite (score 3), possible (score 2), or no (score 0) nutritional implication. Nutritional intake was assessed by asking the parent/caregiver about the subject's recent food intake: no intake at all (score 3), recently decreased or poor intake (score 2), or no change/good intake (score 0). Weights and heights were measured for the anthropometric component of the tool, then the score was determined using the appropriate growth chart (CDC or WHO, based on the child's age). The total score of all 3 elements determined the risk of malnutrition, and was classified into low, medium, and high risk. This tool is accompanied with a care plan based on overall malnutrition risk. If the result was low risk, the STAMP score was not repeated. But if the result was medium risk, the STAMP score was repeated after 3 days, and nutritional intake was monitored for 3 days. If the result was high risk, the

Table 2. The screening tool for the assessment of malnutrition in pediatrics (STAMP) form

Step 1 - Diagnosis				
Does the child have a diagnosis that has any nutritional implications?	Score	1st screening	2nd screeding	3rd screening
Definite nutritional implications	3			
Possible nutritional implications	2			
No nutritional implications	0			
Step 2 - Nutritional intake				
What is the child's nutritional intake?	Score	1st screening	2nd screeding	3rd screening
No nutritional intake	3			
Recently decreased or poor nutritional intake	2			
No change in eating pattern and good nutritional intake	0			
Step 3- Weight and height				
Use a growth chart or the centile quick reference tables to determine the child's measurement	Score	1st screening wt: ht:	2nd screeding wt: ht:	3rd screening wt: ht:
> 3 centile spaces/ ≥ 3 collumns apart (or weight < 2nd centile)	3			
> 2 centile spaces/= 2 collumns apart	1			
0 to 1 centile spaces/collumns apart	0			
Step 4 - Overall risk of malnutrition				
Add up the scores from the boxes in step 1 – 3 to calculate the overall risk of malnutrition	Score	1st screening	2nd screeding	3rd screening
High risk	≥ 4			
Medium risk	2-3			
Low risk	0-1			
Step 5 - Care plan				
What is the child's overall risk of malnutrition, as calculated in step 4?	Use management guidelines and/or local nutritional policies to developed a care plan for the child			
High risk	• Take action • Refer the child to a Dietitian, nutritional support team, or consultant • Monitor as per care plan • Monitor the child's nutritional intake for 3 day			
Medium risk	• Repeat the STAMP screening after 3 days • Amend care plan as required • Continue routine clinical care			
Low risk	• Repeat the STAMP screening weekly while the child is an in-patient • Amend care plan as required			

subject was referred to a nutritional consultant for further action, and STAMP screening was repeated after 3 days.[13] The STAMP form is shown in **Table 2**.

Data were processed and analyzed with *SPSS version 20.0*. The K statistical analysis (a chance-corrected index of agreement) was performed to determine the level of inter-tool agreement between both instruments and anthropometric measurement. Spearman's rank correlation test was used to analyze for a possible correlation between screening tool scores and length of stay. We also calculated the prevalence of HM. Results were considered to be statistically significant for P values < 0.05.

Results

A total of 127 children participated in the study, of whom 70 (55%) were male. Subjects' median age was 11.5 years (range 2.2 to 18.0 years). Their nutritional statuses on admission were mostly normal (55 subjects; 43.3%), while on discharge, most children had mild-moderate malnutrition (56 subjects; 44.1%). Mean length of stay was 8.6 days. According to PNRS, 81 children (63.8%) were at high risk, while 103 children (81.1%) had STAMP scores in the high risk category. The prevalence of HM in this study was 40.9%. The demographic data of subjects is shown in **Table 3**. The largest disease categories were oncology (36.2%) and hematology (23.6%). Only four patients (3.1%) had endocrinological conditions.

Table 3. Subject's demographic data

Characteristics	N=127
Median (range) age, years	11.5 (2.2-180
Gender, n (%)	
Male	70 (55.1)
Female	57 (44.9)
Mean length of stay (SD), day	8.6 (4.60)
Disease category, n (%)	
Oncology	46 (36.2)
Hematology	30 (23.6)
Infection	20 (15.7)
Gastrohepatology	8 (6.3)
Allergy-immunology	7 (5.5)
Surgery	6 (4.7)
Cardiology	6 (4.6)
Endocrinology	4 (3.1)
Nutritional status on admission, n(%)	
Normal	55 (43.3)
Mild-moderate malnutrition	54 (42.5)
Severe malnutrition	8 (6.3)
Overweight	3 (2.4)
Obesity	7 (5.5)
Nutritional status on discharge, n(%)	
Normal	50 (39.4)
Mild-moderate malnutrition	56 (44.1)
Severe malnutrition	11 (8.7)
Overweight	4 (3.1)
Obesity	6 (4.7)
Nutritional risk	
PNRS, n(%)	
Low	2 (1.6)
Moderate	44 (34.6)
High	81 (63.8)
STAMP, n(%)	
Low	1 (0.8)
Medium	23 (18.1)
High	103 (81.1)
Prevalence of HM, n(%)	52 (40.9)

The results of the Kappa statistical test of PNRS and STAMP are explained in **Table 4**. The PNRS score had significant, slight agreement with hospital malnutrition, while the STAMP had not, for identifying hospital malnutrition risk.

Correlations between PNRS and STAMP scores on day one and length of stay are described in **Table 5**.

Table 4. Cross-tabulation of agreement between PNRS, STAMP, and hospital malnutrition

Hospital malnutrition	PNRS		STAMP	
	Low risk*	High risk	Low risk*	High risk
Yes	13	39	7	45
No	33	42	17	58
Sensitivity (%)	92.8		86.5	
Specificity (%)	44.0		22.7	
PPV	0.48		0.43	
NPV	0.71		0.71	
K value	0.175		0.080	
P value	0.028		0.193	

PPV: positive predictive value; NPV: negative predictive value; *Low- and medium-risk categories grouped

Both instruments had positive, but weak correlations with length of stay, indicating that higher PNRS or STAMP scores on day 1 were predictive of longer length of stay.

The associations between disease category and nutritional risk or HM are described in **Table 6**. Disease category was associated with nutritional risk based on PNRS and STAMP scores ($P < 0.05$), but not with HM ($P > 0.05$). The prevalence of HM was highest in the surgery group (83.3%).

Table 5. Correlation between PNRS or STAMP scores on day 1 and length of stay

Score on day 1	Length of stay (r)*	P value
PNRS	0.218	0.014
STAMP	0.188	0.034

*Spearman's rangk correlation test

Table 6. Association between disease category with nutritional risk and HM

Disease category	n	PNRS		STAMP		HM n(%)	P value		
		Low-moderate	High	Low-medium	High		PNRS	STAMP	HM
Oncology	46	4	42	6	40	24 (52.2)			
Hematology	30	15	15	11	19	8 (26.7)			
Infection	20	9	11	2	18	9 (45.0)			
GH	8	5	3	1	7	2 (25.0)			
Allergy-immunology	7	0	7	0	7	1 (14.3)	0.0001*	0.043*	0.100*
Surgery	6	3	3	2	4	5 (83.3)			
Cardiology	6	6	0	0	6	2 (33.3)			
Endocrinology	4	4	0	2	2	1 (25.0)			

HG=gastro-hepatology, *Wilcoxon rank sum test

Discussion

Hospital malnutrition is a health problem of worldwide concern, even in developed countries. The prevalence of HM found in our study was 40.9%, which exceeded the prevalence in two previous Indonesian studies, 24.3% in Malang,[5] and 24.8% in Bali.[6] The difference may have been caused by different ages of subjects. In our study, subjects were children aged 2 to 18 years, while in the Bali study subjects were aged 2 months to 12 years. A French study also reported a 45% HM prevalence.[7] The risk of malnutrition increases if nutritional status upon hospital admission is already compromised. Indeed this was the case in our study, as five out of eight children (62.5%) who came with severe malnutrition were classified to have HM on discharge. The largest proportions of disease in our subjects were oncology and hematology.

Nutritional risk can be identified on admission by applying instruments and scoring based on factors considered to contribute to hospital malnutrition. Five instruments have been developed and validated during the past ten years. The *Screening Tool for Assessment of Malnutrition in Pediatrics* (STAMP) and *Pediatric Nutritional Risk Score* (PNRS) were among those instruments. Anthropometric measurements are used to assess nutritional status worldwide. In terms of agreement with anthropometric measurements, we found that PNRS scores had a significant, slight agreement ($K=0.175$; $P<0.05$) with anthropometric measurements, while STAMP had not ($K=0.080$; $P>0.05$). These results suggest that PNRS had better agreement with anthropometric measures, and can be used to promptly identify children with nutritional risk. A previous study in Bali also validated PNRS for accuracy against anthropometric measures, with sensitivity 79% and specificity 71%.[6] To date, there is no accepted gold standard for screening malnutrition risk in hospitalized children, therefore, we sought to validate the tools by analyzing the inter-tool agreement with Kappa test. The numbers of children classified as high risk using both instruments were quite high, 81 children (63.8%) with PNRS and 103 (81.1%) with STAMP. As the nutritional screening tools were intended for early detection and to prevent HM, higher sensitivity is better even at the expense of low specificity. As such, the tools tend to classify children as high risk at the beginning, but not all "high

risk children" became HM on discharge, probably because nutritional intervention has been done.

In our study, both instruments showed only slight agreement with anthropometric measurements (K< 0.2) for determining nutritional risk. Similarly, a study which compared four screening tools (STAMP, PNRS, STRONGkids, and PYMS) with anthropometric measurements showed substantial inter-tool agreement between those four instruments (K>0.7), but slight agreement with anthropometric measurements (K<0.1).[14] Another study in the United Kingdom (UK) compared three screening tools (PYMS, STAMP, and SGNA) with full dietitian assessment by an independent dietitian as the gold standard. They found that inter-rater agreement for PYMS, STAMP, and SGNA were 0.51, 0.34, and 0.24, respectively.[15] The UK study found that STAMP had fair agreement with a full dietitian assessment. We found that STAMP had no agreement (K=0.080; P > 0.05) with anthropometric measurement. In contrast to the UK study, we used anthropometric measurement as the reference standard to evaluate the screening tools, while the UK study used assessment by dietitian as the gold standard. This difference may have led to differing Kappa value results. In addition, we found that STAMP had sensitivity 86.5% and specificity 22.5%, In agreement with a study at Hasan Sadikin Hospital, Bandung, where STAMP and STRONGkids were compared to SGNA as the gold standard. Wonoputri et al. found STAMP to have a high sensitivity of 100% and specificity of 11.54%.[16] They also evaluated Kappa value of the three instruments against anthropometric measures, and found that STAMP had slight agreement (K=0.018; 95%CI 0 to 0.140) for acute malnutrition, and no agreement (K=0; 95%CI 0 to 0.140) for chronic malnutrition.

We found a high rate of HM in surgery patients, where five out of six children in the Surgery Ward suffered from HM (83.3%). Those five children were admitted for digestive surgical procedures, such as colostomy closure, so they were required to fast before and after the procedure, leading to significant decrease of body weight. The patients were ordered to take nothing by mouth while parenteral nutrition management was inadequate, potentially leading to HM.[2] Children at high nutritional risk according to PNRS were in the oncology and allergy-immunology groups, while STAMP identified children in the cardiology and allergy-immunology groups as high risk. Similarly, a UK study assessed two instruments (STAMP and STRONGkids) and described the distribution of nutritional risk by disease category. Cardiology and respiratory disease were in the high risk group.[17] The explanation for these results is the classification of clinical diagnosis that has nutritional implications according to STAMP, and disease pathology according to PNRS, as both put cardiology in moderate risk, but in STAMP there is an anthropometric component where weight and height measurements are plotted to the growth chart. A previous study found that children with cardiology disease often had growth faltering and malnutrition.[18] This component leads to subjects in the cardiology group scoring higher with STAMP, but not with PNRS.

Positive correlations between PNRS and STAMP scores on day one and length of stay were observed (r=0.218 for PNRS and r=0.188 for STAMP; P<0.05), as described in **Table 5**. Higher PNRS or STAMP scores on the first day of admission are predictive of longer length of stay. Length of hospital stay has a significant impact on overall health-care costs.[19] The cost to treat a nutritionally-at-risk patient is 20% higher than the average cost for a patient with a similar disease/condition, but without nutritional risk.[20]

The strengths of this study are that it provides new information on the prevalence of HM and risk of under-nutrition in a prospectively recruited group of hospitalized children in Medan, North Sumatera. The study was well-accepted by children and their parents and there was consistent assessment of every subject recruited. The limitations of the study were, first, the heterogenity of disease categories had an imbalanced proportion of subjects that may have affected the results. Other previous studies included subjects with one specific diagnosis, in order to validate the screening tools. Second, the implementation of the screening tools was done by a single physician investigator, and not compared to another observer. A good screening tool must be applicable for healthcare staff other than physicians, such as nurses or nutritionists. Further study is needed to evaluate the ease of use of these instruments by healthcare staff, and their effectiveness after being

performed routinely, with the hope of decreasing the prevalence of HM and overall health-care costs. In Haji Adam Malik Hospital, there are no guidelines for nutritional management between high risk and low-moderate risk patients, because neither tool is put into routine use.

In conclusion, PNRS score has slight agreement with anthropometric measurements, therefore PNRS can be used as routine screening tool to identify HM risk in children. However, many other aspects need to be considered before using such tools, including clinical performance, staff workload, and practicality. By knowing the HM risk, a *Nutritional Support Team* (NST) can design the appropriate interventions to prevent HM.

Conflict of Interest

None declared.

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Original Article

Using N-terminal pro-B-type natriuretic peptide to diagnose cardiac abnormalities in children with dyspnea

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Abstract

Background Malnutrition in hospitalized children has negative Background Dyspnea can be caused by various conditions, one of which is cardiac abnormality. Physical examination alone is not sufficient for distinguishing breathing ailments caused by heart abnormalities, especially in small children. N-terminal pro-B-type natriuretic peptide (NT-proBNP) has been used as a marker of heart disease.

Objective To evaluate the usefulness of NT-proBNP levels as a screening tool to diagnose cardiac abnormalities in children presenting with dyspnea.

Methods A cross-sectional study was conducted from August to October 2015 in pediatric patients aged 1 month to 18 years presenting with dyspnea in the Pediatric Ward, Mohammad Hoesin Hospital, Palembang, North Sumatera. All subjects provided blood specimens for NT-proBNP examinations and underwent echocardiography to assess for the presence of cardiac abnormalities. The diagnostic value was analyzed by ROC curve. We determined the optimal cut-off point, sensitivity, and specificity.

Results Fifty-eight subjects, with median age 9.5 (range 1-180) months, consisted of 39 children with and 19 children without cardiac abnormalities. Subjects' median NT-proBNP levels were significantly higher in those with cardiac abnormalities than in those without [1,775 (range 189-9,000) pg/mL vs. 759 (range 245-9,000) pg/mL, respectively, (P=0.002)]. In a ROC curve analysis, the AUC value was 0.75, and at the optimal cut-off point of 1,235 pg/ml had sensitivity of 74.4% and specificity of 73.7%.

Conclusion The level of NT-proBNP can be used to screen for cardiac abnormalities in children presenting with dyspnea. **[Paediatr Indones. 2017;57:124-8 doi: http://dx.doi.org/10.14238/pi57.3.2017.124-8]**.

Keywords: *NT-proBNP; dyspnea; cardiac abnormality*

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a hormone secreted primarily by ventricular cardiac myocytes in response to pressure and volume overload.[1] This hormone has been shown to be an accurate marker for heart disease and helpful in differentiating between heart disease and systemic diseases of the respiratory system in acute conditions. In infants and small children with dyspnea, it is particularly difficult to differentiate between heart failure or other diseases and the etiology.1 A previous study assessed the differences of NT-proBNP levels in healthy children vs. patients with heart failure and other diseases.[2] In addition, another study assessed the benefits of NT-proBNP examination to screen for children with congenital heart disease.[3]

In children, cardiac abnormalities, especially structural abnormalities, are major risk factors of heart failure. The main clinical manifestation most commonly found in children is dyspnea. Dyspnea

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can vary from tachypnea to respiratory distress. In acute conditions, it is often difficult to detect cardiac involvement in children with dyspnea, especially in infants and small children, so misdiagnoses happen frequently, leading to postponed management and poorer prognoses.[4]

The current diagnostic examination to assess for cardiac abnormalities is echocardiography. This test has limitations, as it is generally not available in district hospitals, and it is done by a cardiologist. Therefore, NT-proBNP testing could potentially be used mainly in hospitals with less access to echocardiography, in order to check for heart involvement in children who experience shortness of breath. As such, the initial management of the patient may not be hampered and outcomes can hopefully be improved.

Methods

A cross-sectional study was conducted in the Pediatric Ward of Dr. Mohammad Hoesin Hospital, Palembang, South Sumatera, from August to October 2015. Subjects were recruited by consecutive sampling. Inclusion criteria were patients with dyspnea over the age of 28 days to less than 18 years. Subjects' parents provided written informed consent. Subjects with obesity were excluded. The study was approved by the Committee for Medical Research Ethics, Sriwijaya University Faculty of Medicine. A total of 58 subjects were enrolled in this study. Patients with dyspnea underwent history-taking, physical examinations, and any other procedures in order for a a diagnosis to be made.

Dyspnea was defined to be breathing problems, as judged by an increased respiratory rate above the normal frequency, according to age. Normal limits were defined as: <60 breath per minute (bpm) for < 2 month-olds, <50 bpm for 2 – 12 month-olds, <40 bpm for 1-5-year-olds, and <30 bpm for 5–18-year-olds. Cardiac abnormality was defined as heart failure, congenital heart disease, or acquired heart disease, found on echocardiography examination. Echocardiography, the gold standard for diagnosing cardiac abnormalities, was performed by a pediatric cardiologist on the day blood specimens were obtained. Subjects were classified into two groups, those with and without cardiac abnormalities.

Venous blood specimens (3 mL) were obtained from the subjects and stored in heparinized tubes. Specimen stability was a maximum of 8 hours at room temperature. The *Roche Cardiac proBNP+ and Cobas h 232* instrument was used to determine NT-proBNP levels in 150μL serum by an immunoassay method as per instructions of the manufacturer. Laboratory results were expressed in picogram per milliliter (pg/mL).

Statistical analysis was performed using *PASW 18.0 for Windows* software. Data are presented as median, minimum, and maximum because NT-proBNP levels were non-normally distributed. The Mann-Whitney U test was used for comparisons between groups, and a receiver-operating characteristic (ROC) curve was used to obtain area under the curve (AUC), and a cut-off point with optimal sensitivity and specificity.

Results

Subjects were predominantly female (60.3%) and mostly in the 2 to 12 month age group (43.1%). There were no significant differences in sex, age, or nutritional status distribution between the two groups. In general, the nutritional status of subjects was mostly classified as well-nourished (58.6%). Although severely undernourished subjects were found only in the cardiac abnormality group, the overall proportion of nutritional status between the two groups was not significantly different (P=0.141). The median age of all subjects was 9.5 months (range 1-180). Characteristics data of subjects are presented in **Table 1**.

Subjects with cardiac abnormalities were grouped into congenital heart disease or acquired heart disease. In the congenital heart disease group, the most common diagnosis was ventricular septal defect (VSD) in 12 subjects, whereas in the acquired heart disease group, the most common diagnosis was rheumatic heart disease (RHD) in 4 subjects. The distribution of diagnoses and subjects' median NT-proBNP levels are shown in **Table 2**.

Comparison between subjects with and without cardiac abnormality (P=0.002); between congenital and acquired heart diseases (P=0.011)

Median NT-proBNP levels were significantly

Table 1. Characteristics of subjects

Characteristics	With cardiac abnormality (n=39)	Without cardiac abnormality (n=19)	Total N=58	P value
Gender				
Male	13 (33.3)	10 (52.6)	23 (39.7)	0.131
Female	26 (66.6)	9 (47/4)	35 (60.3)	
Nutritional status, n(%)				
Severely	7 (17.9)	0	7 (12.1)	0.141
undernourished	11 (28.2)	6 (31.6)	17 (29.3)	
Undernourished	21 (53.9)	13 (68.4)	34 (58.6)	
Well-nourished				
Age group, n(%)				
1-2 mo	3 (7.7)	3 (15.8)	6 (10.3)	0.571
>2-12 mo	16 (41.0)	9 (47.4)	25 (43.1)	
>1-5 yr	9 (23.1)	2 (10.5)	11 (19.0)	
>5-15 yr	11 (28.2)	5 (26.3)	16 (27.6)	
Median age (range), mo	12 (1-174)	6.5 (1.5-180)	9.5 (1-180)	

Table 2. Median NT-proBNP levels in subjects with and without cardiac abnormalities

Diagnoses	n	Median NT-proBNP (range), pg/mL
With cardiac abnormalitiy	39	1,775 (189-9,000)
Congenital heart disease	28	1,617 (189-9,000)
Acquired heart disease	11	2,989 (1,251-9,000)
Without cardiac abnormalitiy		
Bronchiolitis	19	759 (245-2,996)
Bronchopneumonia	1	651
Bronchopneumonia with	7	972 (717-2,996)
complications	5	707 (249-1,959)
Asthma bronchiale	2	502 (245-759)
Malignancy	2	1,040 (571-1,509)
Meningitis	2	739 (259-1,219)

higher in subjects with cardiac abnormalities [1,775 (range 189-9,000) pg/mL] compared to subjects without cardiac abnormalities [759 (range 245-2,996) pg/mL] (P=0.002). **Figure 1** shows the comparison of median NT-proBNP levels between the two groups. The ROC curve analysis (**Figure 2**) showed that NT-proBNP performed well in differentiating subjects with and without cardiac abnormalities who present with dyspnea (AUC 0.75). The optimal cut-off of 1,235 pg/mL gave the highest diagnostic accuracy based on ROC curve analysis, with sensitivity of 74.4% and specificity of 73.7%.

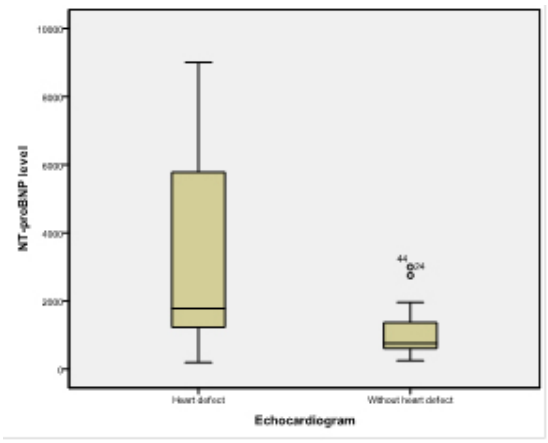

Figure 1. Median NT-proBNP level comparison between subjects with and without heart defects

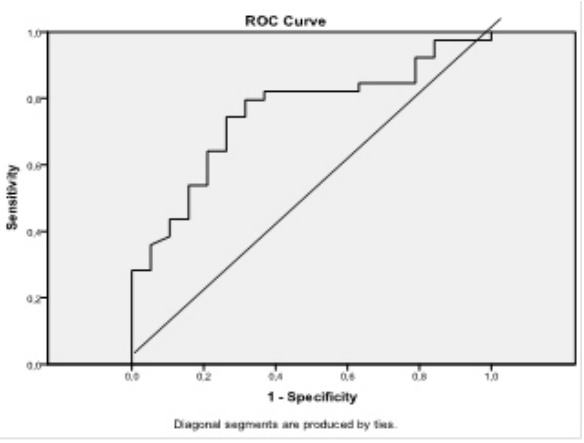

Figure 2. ROC curve for NT-proBNP levels in diagnosing cardiac abnormalities in children with dyspnea

Discussion

The median NT-proBNP level in 39 subjects with cardiac abnormalities was 1,775 (189-9,000) pg/mL. The minimum value, 189 pg/mL, in this group was a subject with a moderate perimembranous outlet (PMO) VSD aged 2 years and 10 months, who had received heart failure treatment during the NT-proBNP examination. In the 19 subjects without cardiac abnormalities, most had bronchopneumonia, with and without complications. The median NT-proBNP level in those without cardiac abnormalities was 759 (245-2,996) pg/mL. In this group, there were two data outliers with values of 2,747 pg/mL and 2,996 pg/mL (maximum). Both of these patients were diagnosed with bronchopneumonia, which caused hypoxia and may have stimulated the production of NT-proBNP.[5] Cohen *et al*. comparing NT-proBNP levels in the groups of healthy children, children with acute lung disease, and children with heart failure. They found that NT-proBNP levels in children with acute lung disease (including pneumonia) were elevated compared to that of healthy children, but the increase was not as high as in the group of children with heart failure.[6]

In our study, median NT-proBNP levels were significantly higher in the cardiac abnormality group than in those without cardiac abnormalites, as diagnosed by echocardiography (P=0.002). Cohen *et al.* found that NT-proBNP levels in children with heart failure were much higher than in those with acute pulmonary diseases and healthy children.6 In addition, Maher *et al.* compared NT-proBNP levels in 30 patients with heart disease to 70 patients with respiratory diseases and infections. They found that NT-proBNP levels in patients with heart disease were much higher than in patients with respiratory disease and infections.[7]

In this study, we assessed the usefulness of NT-proBNP levels as a screening tool to diagnose cardiac abnormalities in pediatric patients who presented with dyspnea. We obtained an AUC value of 0.75, and an optimal cut-off point of 1,235 pg/mL, with sensitivity of 74.4% and specificity of 73.7%. Hammerer-Lercher *et al*. also analyzed NT-proBNP levels retrospectively among 23 children with heart disease and 119 children with non-cardiac diseases (kidneys, lungs, CNS, and others), who were aged over 1 month to 3 years. They reported an AUC value 0.87 and a cut-off point of 2,000 ng/L, with sensitivity 74% and specificity 95%. This study was similar to our study, in terms of variety of cases for comparison, although their age range was smaller (children aged 1 month to 3 years).[8] Their specificity was higher than ours, 95% *vs.* 73.7%, respectively, and our subjects were children with dyspnea, which can be caused by various mechanisms. Furthermore, in most cases dyspnea can also increase NT-proBNP levels. In subjects without cardiac abnormalities, most had bronchopneumonia, which in severe circumstances can also lead to heart failure and increase NT-proBNP levels, even when not accompanied by structural heart abnormalities.[5]

The use of NT-proBNP level as a screening tool to detect congenital heart defects, was made by Moses *et al.* in 2011. They analyzed NT-proBNP levels in 119 children with congenital heart disease and 33 healthy children (with no evidence of abnormalities of the heart), aged 5 days to 12 years. They found that median NT-proBNP levels were 372 (range 60-3000) pg/mL in children with acyanotic congenital heart disease, 1,023 (range 182-3000) pg/ml in those with cyanotic congenital heart disease, and 120 (range 60-380) pg/mL in normal patients. Their AUC value was 0.79, with a cut-off point of 98 pg/mL, with sensitivity 82% and specificity 46%.[3] The study was similar to ours, in terms of subject age and study design. However, Moses *et al*. included pediatric patients admitted to the Cardiology Division at Penang Hospital, Malaysia, who had suspected congenital heart defects based on prior examination at the hospital.[3] We included children who presented with shortness of breath.

Previous studies compared NT-proBNP levels in children with heart disease which had caused heart failure to that of children with lung disease, or to that of a control group of healthy children, and concluded that the hormone was very beneficial in differentiating between the groups.[6-8,16,17] In our study, NT-proBNP levels were compared among children with various diseases who experienced dyspnea. Most of these diseases can also increase NT-proBNP levels.[5,9-15] The cut-off point obtained from our study (1,235 pg/mL) was higher compared that of previous studies that only compared NT-proBNP levels in heart disease with a certain diagnosis.[3,16,17] Additionally, different methods and instruments were used to measure NT-proBNP levels. A previous study reported a cut-off

point of 2,940 pg/mL to distinguish heart disease from pulmonary disease and healthy children, and their AUC was 1.0.6 Another study reported a cut-off point of 415 pg/mL to distinguish children with heart disease from children with non-cardiac illness, and an AUC of 0.958.[16] In addition, Elsharawy et al. reported a cut-off point of 854 pg/ml to distinguish children with VSD who have heart failure from healthy children, with an AUC of 0.98.17 Furthermore, Moses et al. had a cut-off point of 98 pg/ml and AUC of 0.79 to distinguish children with heart defects and those with suspected but unproven heart defects.[3]

In conclusion, NT-proBNP level can be used to diagnose cardiac abnormalities in children who present with dyspnea. Advanced study is still required with a larger sample size of non-cardiac abnormalities, so that NT-proBNP levels can be compared by group with particular organ abnormalities which lead to dyspnea.

Conflict of Interest

None declared.

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Original Article

The immunogenicity and safety of the new Indonesian DTwP-HB-Hib vaccine compared to the DTwP/HB vaccine given with the Hib vaccine

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Abstract

Background Haemophilus influenzae type b (Hib) causes infection with predominant manifestations of pneumonia, meningitis, and other invasive diseases, occurring primarily in children aged under 2 years, particularly in infants. The World Health Organization (WHO) and Indonesian Technical Advisory Group for Immunization recommend to include the Hib vaccine into the national immunization program. The newly developed DTwP-HB-Hib combination vaccine is anticipated to be the preferred choice for Hib vaccine introduction; it is efficient, simple, and has higher coverage.

Objective To evaluate the immunogenicity and safety of a new, combined Bio Farma DTwP-HB-Hib vaccine, compared to the registered Hib monovalent vaccine given simultaneously with the local DTwP-HB vaccine, when used as the primary vaccination of Indonesian infants.

Methods A prospective, randomized, open-label, phase II study was conducted on the DTwP-HB-Hib vaccine compared to the Hib (registered) vaccine given simultaneously with the DTwP-HB vaccine, in Bandung from July 2011 to January 2012. Infants were serially vaccinated at 6-11, 10-15, and 14-19 weeks. Serological assessments were done prior to the first vaccine dose and 28 days after the third dose. Safety was assessed from the time of first injection until 1 month after the last injection.

Results Of 220 healthy infants enrolled, 211 completed the study, with 105 receiving the combined vaccine and 106 the two separate vaccines. All vaccines were well tolerated. No differences in rates of local and systemic reactions were seen between the two methods of administration. No serious adverse events were considered to be related to the vaccines. In the DTwP-HB-Hib primary-vaccination group, at least 98% of the infants reached protective levels of antibodies (seropositivity) against the antigens employed in the vaccines while 96% in the control group.

Conclusion The DTwP-HB-Hib combined vaccine is immunogenic and safe, as well as comparable to the Hib vaccine given simultaneously with to the DTwP-HB vaccine.**[Paediatr Indones. 2017;57:129-37; doi: http://dx.doi.org/10.14238/pi57.3.2017.129-37]**.

Keywords: *DTwP-HB-Hib; Hib; immunogenicity; infants; safety; vaccine*

Before the vaccination era, *Haemophilus influenza* type b (Hib) caused infection with predominant manifestations of pneumonia, meningitis, and other invasive diseases occurring primarily in children aged under 2 years, particularly in infants.[1,2] Pneumonia was responsible for 19% of deaths in children below 5 years of age, of which more than 70% were in Sub-Saharan Africa and Southeast Asia.[2] In Asia, 23% of pneumonia cases were caused by Hib, while other causes were pneumococcus, staphylococcus, streptococcus, and viruses.[3] In Indonesia, pneumonia and meningitis

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caused an estimated 15.5% and 8.8% of all deaths recorded in under-five children, respectively.[4,5]

The WHO has recommended worldwide incorporation of Hib vaccination into all routine infant immunization programs, after 6 weeks of age. A diphtheria-tetanus-pertussis (DTP)-based combination, would be preferable, in order to allow for rapid integration into the existing DTP vaccination schedules.[2] A DTwP-HB vaccine was licensed in Indonesia in 2004 and has been routinely given to infants at 2, 3, and 4 months of age. Phase I of this study showed that the Hib monovalent vaccine was immunogenic and well-tolerated when administered either as a single injection in adults, or in combination (as the DTP-HB-HIB vaccine) in infants, with a one-month interval between doses.[6,7]

The objective of this study was to evaluate the immunogenicity and safety of a new, combined *Bio Farma* DTwP-HB-Hib vaccine, compared to the registered Hib monovalent vaccine given simultaneously with the local DTwP-HB vaccine, when used as the primary vaccination of Indonesian infants according to *Expanded Program on Immunization* (EPI) schedule at 6, 10, and 14 weeks of age, after a birth dose of hepatitis B vaccine, as recommended by the WHO.

Methods

This prospective, randomized, open-label, phase II study of the combined DTwP-HB-Hib vaccine was conducted at three primary health care centers in Bandung from July 2011 to January 2012 and was approved by the Institutional Review Board of Padjadjaran University. Subjects' parents provided written informed consent before enrollment. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines with approval of the Ethics Review Committee and the *National Regulatory Authority* (NRA).

The study population comprised of healthy infants who were 6-11 weeks of age at enrollment, born at 37-42 weeks of gestation, with a birth weight of 2,500-4,000 g, and had received a single dose of monovalent hepatitis B vaccine at 0-7 days after birth, as recorded in their vaccination documentation. Infants were excluded if they had a history of allergic reaction likely to be stimulated by any vaccine component, a history of congenital or acquired immunodeficiency, diphtheria, tetanus, pertussis, hepatitis B, *Haemophilus influenzae* type b infection, uncontrolled coagulopathy or blood disorders, chronic illness, immunosuppressive condition, were undergoing immunosuppressive therapy, had received immunoglobulin therapy or blood products prior to starting or during the study, acute febrile illness at the time of the vaccination, any previous vaccination other than oral polio, BCG vaccine, or HB at birth, or were participating in another clinical study.

We aimed to evaluate the immunogenicity and safety outcomes of the new *Bio Farma* DTwP-HB-Hib vaccine compared to the Hib monovalent vaccine given simultaneously with the DTwP-HB vaccine (DTwP-HB + Hib). At the time of enrollment, subjects were assigned to one of two vaccine groups using a randomized block permutation list.

The study vaccine was a new, liquid DTwP-HB-Hib (pentavalent) vaccine produced by Bio Farma. This vaccine contained 5 antigens. Each 0.5ml dose contained > 30 IU of purified diphtheria toxoid, > 60 IU of purified tetanus toxoid, > 4IU inactivated Bordetella pertussis, 10μg recombinant hepatitis B surface antigen (HBsAg), and 10μg Hib/polyribosyrsibitol phosphate (PRP) conjugated to tetanus toxoid. The DTwP-HB vaccine (*Bio Farma*) contained 4 antigens, with similar amount of antigens, except for hepatitis B (5 μg HBsAg) for each 0.5 mL dose). The Hib monovalent vaccine was imported and already registered in Indonesia. It also contained 10μg Hib/PRP conjugated to tetanus toxoid per dose. Vaccines were administered at 6, 10, and 14 weeks of age, with a 4-week interval between doses. One group received the new DTwP-HB-Hib combination vaccine, while the other group received the DTwP-HB and Hib (registered) vaccines simultaneously. The vaccines were given intramuscularly in the external anterolateral region of the thigh.

Subjects provided 4-mL blood specimens, collected before the first dose of vaccine and 28 days after the third dose, to evaluate antibody responses. Serum specimens were tested for antibodies against all vaccine antigens. Serology assays, except for anti-HBs, were conducted in the *Bio Farma Immunology Laboratory of the Clinical Trial Department*, by technicians who were blinded to group assignment.

Tests for anti-HBs were conducted in a commercial laboratory which had been approved by *Bio Farma's Quality Assurance*.

Antibodies to tetanus and diphtheria were measured by using an enzyme-linked immunosorbent assay (ELISA). An anti-diphtheria and anti-tetanus concentration of >0.01 IU/mL is generally accepted to be the minimum protective threshold, and a concentration of >0.1 IU/mL was regarded to be the standard protective threshold. Pertussis antibodies were measured using a microagglutination assay, with a cut-off dilution of 1/40. An adequate vaccine response was defined to be a post-vaccination antibody titer of four times more than the pre-vaccination titer. Antibodies to hepatitis B surface antigen (anti-HBs) were assayed using a chemiluminescent microparticle immunoassay (CMIA) by AUSAB, Abbott, with a 10 mIU/mL cut-off. Antibodies to PRP were measured by using Improved, Phipps ELISA, to assess serum antibody to Haemophilus influenzae type b. Anti-PRP concentration of ≥ 0.15 μg/mL was generally accepted to be the minimum protective threshold, and a concentration of ≥ 1.0μg/mL was regarded to be the long-term protective threshold.[8]

Safety assessments were conducted by parents and investigators. Study personnel monitored subjects for 30 minutes after each vaccination to detect immediate reactions. Parents were given thermometers and diary cards, and asked to record the occurrence and intensity (mild, moderate, or severe) of local (i.e., pain, redness, swelling, and induration at injection-site), and systemic (e.g., fever [≥38°C] and irritability) reactions, from day 0 through 28 days after each vaccination. For the analyses, adverse events were graded from 1 to 3 in intensity. For local reactions, grade 3 redness, swelling, or induration was defined as areas >5 cm in diameter and grade 3 pain was defined as cried when the leg was moved. For systemic reactions, grade 3 fever was defined as axillary temperature >39°C and grade 3 irritability was defined as inconsolable crying lasting more than three hours. For all other general adverse events, grade 3 was defined as preventing normal daily activities.

Parents of subjects were contacted by telephone three days after each vaccination to ensure completeness of reporting and to screen for adverse events (AEs) requiring medical evaluation or an office visit, an emergency department visit, or hospitalization. Serious adverse events (SAEs) were recorded throughout the study and evaluated by investigators for possible relationships to the study vaccines. At each subsequent visit, the investigator transcribed information from the diary cards onto the Case Report Form, and confirmed other adverse experiences that occurred after the period covered by the diary card.

The minimum required target sample size was established at 220 assessable infants for this study. A 10% dropout rate was anticipated. Data analyses were performed using *SPSS version 18.0* software. Demographic data were expressed as mean (SD) and range. The statistical significance of differences between the vaccine groups in demographic characteristics was assessed by Chi-square test. A P values <0.05 were considered to be an indicator of statistically significant differences between the vaccine groups.

The immunogenicity analyses were performed on the per-protocol population, defined as subjects who received the 3-dose primary series of the appropriately assigned study vaccines, had all blood samples obtained within the time intervals specified in the study protocol, and had a valid post-vaccination serology test result. Antibody seroprotection rates against diphtheria and tetanus toxoids, HBsAg, PRP, and vaccine response rate to pertussis were calculated with 95% confidence intervals (CI). Geometric mean antibody concentration (GMC) with 95% CI were calculated by taking the log-transformation of individual concentration and calculating the anti-log of the mean of these transformed values. Exploratory analyses were performed to compare GMCs and seroprotection rates between the vaccine groups using Mann-Whitney and Chi-square or Fisher's tests.

The safety analyses were based on the intention-to-treat population, defined as all subjects who received at least one dose of vaccine. Exploratory analyses were performed to compare incidences of solicited local and systemic adverse events (any grade intensity) between the vaccine groups using two-sided Fisher's exact test.

Results

Of the 220 infants recruited, 9 did not complete the study protocol due to voluntary withdrawal (3 infants), discontinuation by investigator (3 infants), and disparency with protocol for immunogenicity analyses (3 infants). Investigator excluded 3 infants which dead by severe respiratory failure and severe dehydration (1 infant), had febrile convulsion 3 days after vaccination (1 infant), and had inconsolable crying for more than 3 hours within 3 days (1 infant). The 3 subjects were excluded according to protocol for immunogenicity analyses: 1 due to non-compliance with vaccination procedure (received non-trial vaccine) and 2 due to protocol deviation from the inclusion criteria (different randomization), but these last 2 subjects were not excluded for safety analysis. Hence we had a total of 213 infants in safety analyses, but only 211 subjects in immunogenicity analysis.

The demographic characteristics of subjects are shown in **Table 1**. No clinically significant differences with respect to gender and age were observed between the two groups.

Table 1. Subjec's characteristics

Characteristics	DTwP-HB-Hib (n=105)	DTwP-HB+Hib (n=106)	P value
Gender, n(%)			
Male	47 (44.8)	50 (47.2)	0.832
Female	58 (55.2)	56 (52.8)	
Age, weeks			
Mean (SD)	8.2 (1.5)	8.1 (1.6	0.795
Min-max	6-11	6-11	

Seroprotection and vaccine response rates for each antigen in the study are summarized in **Table 2**. For seroprotection and vaccine response rates, no significant differences were observed between the two groups before and after vaccination with different cut-off values.

Geometric mean concentrations (GMCs) of antibody are presented in **Table 3**. The GMCs before immunization were not significantly different between the two groups for all antigens. After immunization, also not significantly different between the two groups except for anti-HBs. The DTwP-HB-Hib group had significantly higher anti-HBs GMC than the DTwP-HB+Hib group (441.54mIU/mL vs. 213.84 mIU/mL, respectively, P=0.001).

Table 2. Summary of seroprotection rates of antibody concentration

Antibody	Timing of blood collection	Criterion	DTwP-HB-Hib			DTwP-HB+Hib			P value
			N[a]	%SP[b]	95%CI	N	%SP	95%CI	
Diphtheria	Pre-dose 1	≥ 0.01 IU/mL	35	33.3	25.0 to 42.8	44	41.5	32.6 to 51.0	0.228
	Pre-dose 1	≥ 0.1 IU/mL	3	2.9	1.0 to 8.1	3	2.8	1.0 to 8.0	1.00
	Post-dose 3	≥ 0.01 IU/mL	105	100.0	96.5 to 100	106	100.0	96.5 to 100	1.00
	Post-dose 3	≥ 0.1 IU/mL	86	81.9	73.5 to 88.1	88	83.0	74.7 to 89.0	0.831
Tetanus	Pre-dose 1	≥ 0.01 IU/mL	105	100.0	96.5 to 100.0	105	99.1	94.8 to 99.8	1.00
	Pre-dose 1	≥ 0.1 IU/mL	99	94.3	88.1 to 97.4	102	96.2	90.7 to 98.5	0.538
	Post-dose 3	≥ 0.01 IU/mL	105	100.0	96.5 to 100.0	106	100.0	96.5 to 100.0	1.00
	Post-dose 3	≥ 0.1 IU/mL	101	96.2	90.6 to 98.5	104	98.1	93.4 to 99.5	0.445
Pertusis	Pre-dose 1	≥ 40 (1/dil)	7	6.7	3.3 to 13.1	6	5.7	2.6 to 11.8	0.761
	Pre-dose 1	≥ 80 (1/dil)	5	4.8	2.1 to 10.7	4	3.8	1.5 to 9.3	0.748
	Post-dose 3	≥ 40 (1/dil)	94	89.5	82.2-94.0	100	94.3	88.2 to 97.4	0.199
	Post-dose 3	≥ 80 (1/dil)	89	84.8	76.7 to 90.8	95	89.6	82.4 to 94.1	0.291
	Post-dose 3	VRR[c]	90	85.7	77.8 to 91.1	98	92.5	85.8 to 96.1	0.177
Hepatitis B	Pre-dose 1	≥ 10 mIU/mL	19	18.1	11.9 to 26.5	21	19.8	13.3 to 28.4	0.751
	Post-dose 3	≥ 10 mIU/mL	104	99.0	94.8 to 99.8	102	96.2	90.7 to 98.5	0.369
PRP (Hib)	Pre-dose 1	≥ 0.15 µg/mL	30	28.6	20.8 to 37.8	28	26.4	19.0 to 35.5	0.726
	Pre-dose 1	≥ 1.0 µg/mL	14	13.3	8.1 to21.1	17	16.0	10.3 to 24.2	0.579
	Post-dose 3	≥ 0.15 µg/mL	103	98.1	93.3 to 99.5	105	99.1	94.8 to 99.8	0.621
	Post-dose 3	≥ 1.0 µg/mL	101	96.2	90.6 to 98.5	101	95.3	89.4 to 98.0	1.00

[a] N= number of subjects with a valid serology result pre-dose 1 and post-dose 3

[b] %SP= seroprotection rate

[c] VRR (vaccine response rate) was defined as >4 times the pre-vaccination concentration

Table 3. Summary of geometric mean antibody concentration

Antibody	Timing of blood collection	DTwP-HB-Hib		DTwP-HB+Hib		P value
		GMC	95%CI	GMC	95%CI	
Diphtheria	Pre-dose 1	0.040	0.026 to 0.062	0.045	0.029 to 0.068	0.413
	Post-dose 3	0.259	0.209 to 0.321	0.289	0.233 to 0.359	0.543
Tetanus	Pre-dose 1	0.639	0.530 to 0.771	0.685	0.575 to 0.816	0.787
	Post-dose 3	1.147	0.916 to 1.147	1.137	0.894 to 1.446	0.818
Pertusis	Pre-dose 1	6.819	5.799 to 8.019	6.666	5.809 to 7.651	0.607
	Post-dose 3	219.63	160.36 to 300.88	332.81	257.69 to 429.83	0.067
Hepatitis B	Pre-dose 1	2.082	1.508 to 2.876	2.458	1.693 to 3.650	0.661
	Post-dose 3	441.57	347.70 to 560.79	213.84	156.64 to 292.01	0.001
PRP (Hib)	Pre-dose 1	0.988	0.925 to 1.055	1.075	0.9990 to 1.167	0.989
	Post-dose 3	12.612	9.689 to 16.421	11.663	8.962 to 15.81	0.876

GMC=geometric mean concentration

Table 4. Local reaction within 72 hours after each injection

Reactions	DTwP-HB-Hib (1)			DTwP-HB+Hib						P value	
				DTwP-HB site (2)			DTwP-HB site (2)				
	mEv	nSj	%Sj	mEv	nSj	%Sj	mEv	nSj	%Sj	1 vs 2	1 vs 3
After 1st injection											
Local reaction	36	16	14.9	27	19	17.3	14	9	8.2	0.779	0.177
Solicited reactions											
Pain	11	11	10.3	9	9	8.2	7	7	6.4	0.764	0.424
Redness	8	8	7.3	3	3	2.7	2	2	1.8	0.742	0.199
Swelling	10	10	9.3	7	7	6.4	3	3	2.7	0.572	0.077
Induration	7	7	6.5	8	8	7.3	2	2	1.8	0.956	0.098
After 2nd injection											
Local reaction	14	14	13.2	19	11	3.7	14	9	8.3	0.618	0.341
Solicited reactions											
Pain	8	8	7.5	8	8	7.4	6	6	5.6	0.840	0.742
Redness	3	3	2.8	3	3	2.8	3	3	2.8	1.0	1.0
Swelling	3	3	2.8	3	3	2.8	2	2	1.8	1.0	0.680
Induration	0	0	0	5	5	4.6	3	3	2.8	0.060	0.247
After 3rd injection											
Local reaction	7	6	5.7	13	13	12.0	11	11	10.2	0.168	0.341
Solicited reactions											
Pain	1	1	0.9	6	6	5.5	6	6	5.5	0.119	0.119
Redness	2	2	1.9	3	3	2.8	1	1	0.9	1.0	0.618
Swelling	3	3	2.8	2	2	1.8	3	3	2.8	0.680	1.0
Induratio	1	1	0.9	2	2	1.8	1	1	0.9	1.0	1.0

nEv=number of event, nSj=number of subject, %Sj=percentage of subject

Subject's local reactions within 72 hours are presented in **Table 4**, where 1 subject may experience more than 1 local reactions. After the first, second, and third injections, 57 forms of local reactions occurred in 36 subjects (11.3%) in the DTwP-HB-Hib group. The most frequent reaction was pain; other reactions were redness, swelling, and induration. In the DTwP-HB site, 59 local reactions were noted after the injections in 43 subjects (13.3%). The most frequent reaction was pain; other reactions were redness, induration, and swelling. In the Hib site, 39 local reactions were reported after the injections in 29 subjects (8.9%). The most frequent reaction was pain; other reactions were redness, swelling, and induration. In this study, 2 subjects in the DTwP-HB-Hib group site and 1 subject in the Hib site of the DTwP-HB+Hib group

Table 5. Systemic reactions within 72 hours after each injection

Systemic reaction	DTwP-HB-Hib		DTwP-HB+Hib		P value
	nSj	%Sj	nSj	%Sj	
After 1st injection					
Fever (≥ 38°C)	30	28.0	28	25.5	0.274
Irritability	3	2.8	4	3.6	0.121
Others	2	1.8	1	0.9	0.666
After 2nd injection					
Fever (≥ 38°C)	27	25.3	19	17.6	0.444
Irritability	1	0.9	3	2.8	0.500
Others	4	3.8	2	1.8	0.831
After 3rd injection					
Fever (≥ 38°C)	21	20.0	15	13.9	0.049*
Irritability	1	0.9	1	0.9	0.246
Others	5	4.8	2	1.8	0.691

nSj=number of subjects

presented with severe local reactions within 72 hours after each injection, (swelling and induration). The incidence and intensity of symptoms were comparable in both vaccine groups. There was no increase in reactogenicity with doses for local symptoms. Local reactions were low in both groups; most reactions were mild, and resolved spontaneously within the two-day follow-up period. No subjects presented with local reactions between 72 hours and 28 days after each injection.

Subject's systemic reactions within 72 hours are presented in **Table 5**. In the DTwP-HB-Hib group, fever was reported in 28.0%, 25.3%, and 20.0% of subjects after the 1st, 2nd, and 3rd injections, respectively. In the DTwP-HB+Hib group, fever was reported in 25.5%, 17.6%, and 13.9% of subjects after the 1st, 2nd, and 3rd injection, respectively. There were no significant differences between the DTwP-HB-Hib and DTwP-HB+Hib groups with regards to fever after each injection, except after the 3rd injection, with significantly fewer in the DTwP-HB+Hib group (P=0.049). No anaphylactic or other severe reactions were reported within 30 minutes after any dose of vaccines.

During the study, 11 cases of serious adverse events (SAEs) were reported. There was one death during the study due to respiratory failure and septicemia 20 days after the infant received the first combination vaccine dose. The investigators and the *Indonesian National Committee of Adverse Event Following Immunization* did not consider the death to be related either to the vaccination or study procedure. One subject suffered from complex febrile convulsion, classified as a vaccine reaction in field classification and probable in causality assessment, but the patient resolved spontaneously. The remaining 9 SAEs were mainly due to infectious diseases such as bronchopneumonia, diarrhea, and aspiration pneumonia. The children recovered after treatment and hospitalization. All SAE cases were audited by I*ndonesian National Committee of Adverse Event Following Immunization*.

Discussion

As of 2000, the WHO had achieved 90% coverage with the DTP vaccination in infants aged less than one year. In countries with endemic hepatitis B, early infant immunization is recommended. Since the coverage with hepatitis B immunization is much lower in Indonesia, combining it with DTP was thought to be the best way to increase hepatitis B immunization coverage. The first clinical trial of the DTwP-HB vaccine started in April 2002 in three centers, involving about 730 healthy infants from Bogor, Bandung, and Banjar Baru. The trial consisted of 5 groups of subjects, each with different doses of hepatitis B and different schedules of immunization. The immunogenicity and safety of the DTwP-HB vaccine were not significantly different to that of separate administrations of the DTwP and hepatitis B vaccines, which had been commonly used in the Immunization Programme up to that point.[9]

In 1998, the WHO recommended the Haemophilus influenzae type B (Hib) vaccine to be

included in routine infant immunization programs.[2] Due to limited national capacity, the Hib antigen was integrated as a DTP-based combination vaccine. Bio Farma had developed a new, pentavalent, combined diphtheria-tetanus-whole cell pertussis-hepatitis B/Hib (DTwP-HB-Hib) vaccine containing 10 μg of polyribosylribitol phosphate (PRP) conjugated to tetanus toxoid.

The first Hib vaccine was used in a phase I trial of 25 healthy adults, where 1 subject received 1 dose of Hib monovalent vaccine. No serious adverse events followed vaccination. However, pain occurred in 11 subjects and systemic reactions (myalgia) occurred in 5 subjects. Most reactions were mild and disappeared within 24 hours. All subjects (100%) reached protective levels of antibodies (seroprotectivity) against Hib. The GMT increased from 0.68 μg/mL to 30.16 μg/mL.[6] The first clinical trial of the DTwP-HB-Hib vaccine was conducted in April–June 2011, involving 30 pediatric subjects. Eighteen subjects (60%) reported fever within 3 days after the vaccination. Most cases of fever were mild in intensity and resolved within 3 days. Furthermore, no serious adverse events were reported. All subjects had seroprotective antibodies against tetanus, diphtheria, hepatitis B, and Hib.[7]

The main objective of this study was to compare the immunogenicity and safety of the new DTwP-HB-Hib pentavalent combination vaccine to separate injections of DTwP-HB and Hib (DTwP-HB+Hib) vaccines, in a group of infants who had received a dose of hepatitis B vaccine at birth. After the primary series, 100% of subjects in both vaccine groups achieved levels considered to be protective for diphtheria (>0.01U/mL) and tetanus (>0.01 IU/mL). Also, 99% of the DTwP-HB-Hib pentavalent group and 96.2% of the DTwP-HB+Hib group achieved protective levels of hepatitis B (>10 mIU/mL). For pertussis, 89.5% in the DTwP-HB-Hib pentavalent group and 94.3% in the DTwP-HB+Hib group achieved seroprotection of 40 (1/dil). We observed no differences in seroprotection rates between the two groups. We also noted that the Hib response in the DTwP-HB-Hib pentavalent combination group was not significantly different to that of the separately administered monovalent Hib registered vaccine. In our Bandung study of the primary-vaccination three-dose course, 98.1% of the infants in the DTwP-HB-Hib group and 99.1% of the DTwP-HB+Hib group had anti-PRP titers above the conservative threshold of protection (0.15 μg/mL). In addition, 96.2% of those in the DTwP-HB-Hib group and 95.3% of the DTwP-HB+Hib group had titers above 1.0 μg/mL.

A 2009-2010 Indian study in 661 infants aged 6 to 8 weeks, found 100% seroprotection to anti-PRP using pentavalent combination vaccines with a one month-interval between doses.[10] Another study also used pentavalent vaccines at one month-intervals in 608 infants aged 6 weeks and showed anti-PRP results similar to our study: 100% protection for short-term protection (> 0.15μg/mL) and 95% for long-term protection (> 1 μg/mL).[11] Furthermore, another Indian study in 165 infants at 6, 10, and 14 weeks of age found results similar to our study: at one month after the third vaccination, percentages of infants achieving predefined protective antibody levels were 99% diphtheria; 100% tetanus; 98% hepatitis B; 100% Hib short-term (≥ 0.15 μg/mL); 95% Hib long-term (≥ 1.0 μg/mL) protection; and 99% for pertussis (relevant immune response).[12] These three studies were conducted without control groups.

An Ankara, Turkey study in 2003-2004 was conducted in 303 infants 6 weeks of age. Infants received three doses at one month-intervals, of either a combination vaccine or a control DTP-Hib with separate hepatitis B vaccine. Seroconversion of all antigens were similar between the two groups.[13] A Latin American study used pentavalent vaccines in 1,000 infants. Statistical comparisons following the primary vaccination showed that, in terms of the antibody response to the PRP antigen, the combined DTP-HB-Hib vaccine was clinically non-inferior to the licensed DTP-HB and Hib vaccines. Other antigens also showed similar immune responses.[14] In addition, an Indian study of a new, pentavalent vaccine compared it to two other vaccines, the DTP-HB+Hib vaccine (separate injections) and another registered pentavalent vaccine. The authors found that 98.32% of subjects in the vaccine trial group had seroprotective anti-PRP-T IgG antibody concentrations (≥0.15 μg/mL) as compared to 100% and 98.94% of subjects in the DTP-HB+Hib and the other registered pentavalent vaccine groups, respectively. Seroprotective levels for anti-HBs (≥10 mIU/mL) were observed in 97.77%, 97.83%, and 98.94% of subjects in the vaccine trial group, DTP-

HB+Hib, and other registered pentavalent vaccine groups, respectively. Comparable immune responses were observed for the other three components (D, T, and P) in all groups.[15]

Compared to all studies noted above, we found that both our groups had similar results, in terms of immune response, except for anti-HBs. In the DTwP-HB-Hib group, the hepatitis B response reached 99.0% seroprotection after three doses of vaccine, with GMCs of 441.57 mIU/mL, compared to a 96.2% seroprotection rate, with GMCs of 213.84 mIU/mL in the DTwP-HB+Hib group. Although the seroprotection rate was not significantly different, the GMCs were (P= 0.001), perhaps because of differing doses. The HBsAg in DTwP-HB was only 5μg/dose (according to Indonesian immunization policy at that time for DTwP-HB vaccine), while the HBsAg in DTwP-HB-Hib was 10μg/dose, according to the international regulation for hepatitis B vaccines.

After the first, second, and third injections, local reactions were seen in 14.9%, 13.2%, and 5.7% of infants at the DTwP-HB-Hib site, 17.3%, 3.7%, 12.0% at the DTwP-HB site, and 8.2%, 8.3%, 10.2% at the Hib site, respectively. Local reactions classified as severe were seen in only two subjects from the DTwP-HB-Hib sites (swelling & induration) and one subject from the Hib site (swelling), after the first injection. Pain at the injection site was the most commonly reported local reaction. Both forms of administration produced comparable and acceptable rates of local reactions. Fever was the most frequent systemic event. In the DTwP-HB-Hib group, fever was reported in 28.0%, 25.3%, and 20.0% of subjects, and in the DTwP-HB+Hib group fever was reported in 25.5%, 17.6%, and 13.9% of subjects after the 1st, 2nd, and 3rd injection, respectively. There were no significant differences in rates of fever between vaccine groups, except after the 3rd injection (P=0.049). Most systemic events were mild in severity at all three doses. Another systemic event, irritability, was very rare, only 1 to 4 subjects in each group. One subject had a complex febrile convulsion, classified as a vaccine reaction in field classification and probable in causality assessment, but it resolved spontaneously.

An Indian study also found pain to be the most frequent local reaction, with 29%, 18%, and 10%, after the 1st, 2n^{d}, and 3rd injections, respectively. Severe local reaction occurred only as pain in 5%, 4%, and 5% of subjects, after the 1st, 2nd and 3rd injections, respectively. Fever was found in 21%, 18%, and 15%, after the 1st, 2nd and 3rd injections, respectively.[12] Rao *et al.* compared a new pentavalent vaccine to the DTP-HB+Hib vaccine (separate injection) and another registered pentavalent vaccine. They also found that pain was the most frequent local reaction in all groups, with 35.54%-36.26% of subjects. Severe local reactions were found in all groups for swelling, with 17.42%-20.21%. Fever was only found to be 5.68%-7.09%. The most frequent systemic event was crying, in 23.34%-25.89%.[15] Compared to other studies, our trial vaccine induced fewer local reactions, but for systemic events, a higher percentage of fever, and fewer other systemic events than other pentavalent vaccines. According to the WHO information sheets for DTP-based vaccines, fever > 38°C and irritability may occur 45-75% of vaccinees, much higher than our findings.[16]

In our study, one child had a complex febrile convulsion, classified as a vaccine reaction. According to WHO information sheets for DTP-based vaccines, febrile seizure may occur in 60 cases out of 100,000 doses. Barlow et al. reported that the risk of febrile seizure may be increased only on the day of the DTP-based immunization, with a relative risk of 5.7.[17] Likewise, Sun et al. reported that the risk of febrile seizure may be increased after DTaP immunization, with relative risk of 6.02 on the first day and decreasing to 3.94 on the second day.[18]

In conclusion, the DTwP-HB-Hib combined vaccine is immunogenic and safe, as well as comparable to the Hib vaccine given simultaneously with the DTwP-HB vaccine.

Conflict of Interest

None declared.

Acknowledgments

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Original Article

CD4+ T-cell, CD8+ T-cell, CD4+/CD8+ ratio, and apoptosis as a response to induction phase chemotherapy in pediatric acute lymphoblastic leukemia

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Abstract

Background Acute lymphoblastic leukemia (ALL) is a neoplastic disease resulting from somatic mutation in the lymphoid progenitor cells, often occuring in children aged 2-5 years, predominantly in males. Results from the induction phase of chemtherapy are used to measure success, but the failure remission rate is still high. Increased apoptosis of cancer cells, as induced by $CD4^+$ and $CD8^+$ T-cells, is an indicator of prognosis and response to chemotherapy.

Objective To assess for correlations between $CD4^+$, $CD8^+$, or $CD4^+/CD8^+$ ratio to the chemotherapy induction phase response (i.e., apoptosis) in pediatric ALL patients.

Methods This observational analytical cohort study was done in 25 pediatric ALL patients. Whole blood (3 mL) with EDTA anticoagulant were used to measure absolute counts of $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ ratio. Peripheral blood mononuclear cells (PBMC) were examined for apoptosis. The principle of $CD4^+$, $CD8^+$ examination was bond between antigens on the surface of the leukocyte in the blood with fluorochrome labeled antibodies in the reagents, while the principle of apoptosis examination was FITC Annexin V will bonds with phosphatidylserine that moves out of the cell when the cell undergoes apoptosis, then intercalation with propidium iodide (PI). All examination were detected by flow cytometry BD FACSCalibur.

Results Subjects were 25 newly-diagnosed, pediatric ALL patients (64% males and 36% females). Most subjects were 3 years of age (20%). Numbers of $CD4^+$ and $CD8^+$ cells, as well as $CD4^+/CD8^+$ ratio were significantly decreased after chemotherapy. However, apoptosis was not significantly different before and after chemotherapy (P=0.689), There were significant negative correlations between apoptosis and $CD4^+$ (P=0.002; rs=-0.584), and $CD8^+$ (rs=-0.556; P=0.004), before chemotherapy. In addition, $CD4^+$-delta and apoptosis-delta also had a significant positive correlation (rs=0.478; P=0.016). However, no correlation was found between the $CD4^+/CD8^+$ ratio and apoptosis, before or after chemotherapy.

Conclusion There are significantly lower mean $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ ratio after chemotherapy than before. Also, there are significant correlations between $CD4^+$-delta and apoptosis-delta, as well as between apoptosis and $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ ratio, before chemotherapy. The $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ ratio can be used to predict apoptosis before chemotherapy. In addition, $CD4^+$-delta can be used to predict apoptosis-delta as a response to induction phase chemotherapy in pediatric ALL. **[Paediatr Indones. 2017;57:138-43 doi: http://dx.doi.org/10.14238/pi57.3.2017.138-43]**.

Keywords: *ALL; CD4+; CD8+; apoptosis*

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Acute lymphoblastic leukemia (ALL) is a neoplastic disease resulting from somatic mutation in the lymphoid progenitor cells in the bone marrow, often occurring in children aged 2-5 years, and predominantly in males. Diagnosis of ALL is based on finding ≥ 25% lymphoblasts of blood smear or bone marrow aspiration (BMA) evaluation. Outcome criteria was made as follows, < 5% lymphoblasts was belonging to remission, and > 5% was belonging to non remission, including partial remission.[1-5]

Failure of apoptosis and the immune response causes uncontrolled growth of cancer cells. The progression of ALL has been correlated with quantitative and qualitative (function) host immune responses. Cluster differentiation 8$^+$ (CD8$^+$) T-cell are an effector cell resulting an apoptosis tumor cells by cytotoxic or cytolytic effects. Anti-tumor mechanisms of CD4$^+$ T-cells are not fully understood. The CD4$^+$ T-cells have no cytotoxic or cytolytic effects. Many cytokines produced by CD4$^+$ T-cells are needed for the development of CD8$^+$ effector cells. Tumor necrosis factor (TNF) and interferon-gamma (IFN-gamma) secreted by activated CD4$^+$ T cells will induce expression of major histocompatibility complex I (MHC I) and induce CD8$^+$ T-cell activity to lyse tumor cells.[6-10] The induction phase of chemotherapy is usually used as a measure of successful chemotherapy, but remission failure rates are still high.[10] Increased apoptosis of cancer cells induced by CD4$^+$ or CD8$^+$ T-cells can be used as one indicator of prognosis and response to induction phase chemotherapy in pediatric ALL patients.[9] The aim of this study was to assess for correlations between apoptosis and CD4$^+$, CD8$^+$, and CD4$^+$/CD8$^+$ ratio, before and after the induction phase of chemotherapy in pediatric ALL patients.

Methods

This observational, analytical, cohort study included 25 new cases of pediatric ALL patients who were diagnosed based on bone marrow aspiration at the Department of Pediatrics, Airlangga University Medical School/Dr. Soetomo Hospital, Surabaya, from July to December 2016. The inclusion criteria were new cases of pediatric ALL aged 1 month to 16 years who had never received chemotherapy, and planned to undergo induction phase chemotherapy regularly for 6 weeks. The exclusion criteria were ALL patients with a history of HIV/AIDS or had infections as indicated by fever and other signs. Healthy patients control were obtained from healthy volunteers aged 1 month to 16 years who had investigated no history of certain diseases and seem good condition during the examination was done. This study was approved by the Ethics Committee of our institution and written informed consent was obtained from all patients' parents.

Clinical characteristics including age, sex, hemoglobin (Hb) level, platelet count, and leukocyte count were obtained at diagnosis (before chemotherapy) and after induction phase chemotherapy. Whole blood (3 mL) with EDTA anticoagulant were used to measure absolute counts of CD4$^+$, CD8$^+$, and CD4$^+$/CD8$^+$ ratio. Reagents used for CD4$^+$, CD8$^+$ examination was BD Multitest CD3FITC/CD8PE/CD45PerCP/CD4APC. The principle of CD4$^+$, CD8$^+$examination was to detect bonds between antigens on the surface of the leukocyte present in the blood with fluorochrome labeled antibodies present in the reagents. Peripheral blood mononuclear cells (PBMC) were examined for apoptosis. The FITC Annexin V and propidium iodide (PI) were used for apoptosis examination. The principle of apoptosis examination was FITC Annexin V will bonds with phosphatidylserine that moves out of the cell when the cell undergoes apoptosis, then intercalation with propidium iodide (PI). The FITC Annexin V can identify early apoptosis, late apoptosis and necrosis based on nucleus changes. Propidium Iodide (PI) was used to distinguish late apoptosis and necrosis with early apoptosis. All examination were detected by flow cytometry BD FACSCalibur performed before and after induction phase chemotherapy, at the Department of Clinical Pathology, Airlangga University Medical School/ Dr. Soetomo Hospital, Surabaya.

Comparison of Hb levels before and after induction phase chemotherapy was evaluated by paired T-test, while leukocyte counts, platelet counts, CD4$^+$, CD8$^+$ cells, CD4$^+$/CD8$^+$ ratio, and apoptosis before and after induction phase chemotherapy were evaluated by Wilcoxon Signed Rank test. Correlations of absolute count of CD4$^+$, CD8$^+$ cells, CD4$^+$/CD8$^+$ ratio with apoptosis were evaluated by Spearman's

correlation test. $CD4^+$ -delta was defined as difference between number of $CD4^+$ before and after induction phase chemotherapy, as well as $CD8^+$-delta, $CD4^+$/$CD8^+$-delta, and apoptosis delta. Results with P values ≤ 0.05 were considered to be statistically significant.

Results

During the study period, 41 new cases of pediatric ALL patients fulfilled the inclusion criteria, but 16 patients dropped out, of whom 11 patients died and 5 patients did not complete the induction phase of chemotherapy. Hence, our cohort study of 25 new cases of pediatric ALL patients consisted of 16 males (64%) and 9 females (36%), with a 1.78:1 ratio (**Table 1**). Many of our subjects were 3 years of age (20%). Leucocyte count was significantly lower (P=0.014) but platelet count was signigficantly higher (P=0.000), between before and after induction phase of chemotherapy. Mean numbers of $CD4^+$ and $CD8^+$ cells, as well as $CD4^+/CD8^+$ ratio were significantly decreased after induction phase chemotherapy. Mean numbers of $CD4^+$ before induction phase chemotherapy was 3,060.24 (SD 4660.03), after induction phase chemotherapy was 887.64 (1531.33). Mean numbers of CD8+ before induction phase chemotherapy was 3,084.76 (SD 4535.51), after induction phase chemotherapy was 1,647.28 (SD 3644.99). Mean numbers of $CD4^+/CD8^+$ ratio before induction phase chemotherapy was 1.12 (SD 0.67), after induction phase chemotherapy was 0.65 (SD 0.61). However, apoptosis did not significantly decrease after chemotherapy (P=0.689), before induction phase chemotherapy was 18.19 (SD 19.82) and after induction phase chemotherapy was 14.09 (SD 10.85) (**Table 2**).

Table 1. Clinical characteristics of study subjects before and after induction phase chemotherapy

Characteristics	N=25	P value
Gender, n		
Male	16	
Female	9	
Age, n		
1-5 years	13	
6-10 years	7	
≥ 10 years	5	
Leucocyte count, $x10^3$ µL		0.014*
Before		
Mean (SD)	52.68 (120.45)	
Median (range)	8.81 (1.20 – 585.00)	
After		
Mean (SD)	7.58 (10.78)	
Median (range)	5.12 (0.79 – 56.00)	
Hb concentration, g/dL		0.165
Before		
Mean (SD)	10.30 (3.13)	
Median (range)	9.48 (5.00 – 16.00)	
After		
Mean (SD)	11.35 (2.02)	
Median (range)	11.30 (7.60 – 15.40)	
Thrombocyte count, $x10^3$ µL		0.000*
Before		
Mean (SD)	62.26 (54.17)	
Median (range)	42.6 (5.00 – 204.00)	
After		
Mean (SD)	190.33 (119.07)	
Median (range)	177.00 (18.80 – 447.00)	

*significant if P ≤ 0.05, Before=before induction phase chemotherapy, After=after induction phase chemotherapy

Table 2. Comparison between $CD4^+$, $CD8^+$ cells, $CD4^+/CD8^+$ ratio, and apoptosis, before and after induction phase chemotherapy in pediatric ALL patients

Variables	Time	Mean (SD)	Median (range)	P value
$CD4^+$, cells	Before	3,060.24 (4660.03)	1751.00 (210.0 – 23,016.0	0.000*
	After	887.64 (1531.33)	199.00 (4.0 – 5,966.0)	
$CD8^+$, cells	Before	3,084.76 (4535.51)	1820.00 (341.0 – 18,541.0)	0.004*
	After	1,647.28 (3644.99)	423.00 (52.0 – 17,859.0)	
$CD4^+/CD8^+$ ratio	Before	1.12 (0.67)	1.03 (0.33 – 2.91)	0.004*
	After	0.65 (0.61)	0.44 (0.06 – 2.31)	
Apoptosis	Before	18.19 (19.82)	10.83 (0.26 – 80.58)	0.689
	After	14.09 (10.85)	10.34 (2.86 – 49.17)	

*significant if $P \leq 0.05$, Before=before induction phase chemotherapy, After=after induction phase chemotherapy

Table 3. Comparison of apoptosis between pediatric ALL patients and healthy children before and after induction phase chemotherapy

Variables	Apoptosis		P value
	Mean (SD)	Median (range)	
ALL patients (before chemotherapy)	18.19 (19.82)	10.83 (0.26-80.58)	0.683
Healthy control patients	16.21 (3.52)	17.73 (12.19-18.71)	
ALL patients (after chemotherapy)	14.09 (10.85)	10.34 (2.86-49.17)	0.316
Healthy control patients	16.21 (3.52)	17.73 (12.19-18.71)	

Table 4. Correlation between apoptosis and CD4+, CD8+ cells, and CD4+/CD8+ ratio before and after induction phase chemotherapy

Variables	Correlation with apoptosis before chemotherapy		Correlation with apoptosis after chemotherapy	
	r_s	P value	r_s	P value
$CD4^+$ cells	- 0.584	0.002	0.081	0.701
$CD8^+$ cells	- 0.556	0.004	- 0.105	0.619
$CD4^+/CD8^+$ ratio	- 0.117	0.579	0.040	0.849

A comparison of apoptosis was done in ALL patients and healthy control subjects. We found no significant differences in apoptosis between the two groups either before or after chemotherapy (**Table 3**).

We also assessed for correlations between apoptosis, before and after chemotherapy, and $CD4^+$, $CD8^+$ absolute counts, and $CD4^+/CD8^+$ ratio. There were significant, negative correlations between apoptosis and $CD4^+$, as well as apoptosis and $CD8^+$, before chemotherapy. However, there was no significant correlation between $CD4^+/CD8^+$ ratio and apoptosis, before or after chemotherapy (**Table 4**).

There was a significant correlation between $CD4^+$-delta and apoptosis-delta after induction phase chemotherapy. However, we observed no significant correlation between apoptosis-delta and $CD8^+$-delta or $CD4^+/CD8^+$-delta (**Table 5**).

Discussion

Acute lymphoblastic leukemia is a hematological malignancy that often occurs in children, and comprises 25–30% of all pediatric malignancies. The highest incidence is in 2–5-year-olds, and predominantly in boys. Our cohort study of 25 new cases of pediatric ALL patients consisted of 16 (64%) males and 9 (36%) females, with 1.78 : 1 ratio. Many subjects were 3 years of age (20%) (**Table 1**).

There were significant differences in leukocyte (P=0.014) and thrombocyte (P=0.000) counts, before and after induction phase chemotherapy. Uncontrolled lymphocyte proliferation and defective apoptosis in ALL patients caused leukocytosis dominated by lymphoblasts. Infiltration of hematopoetic cells by leukemic cells accumulated in the bone marrow

causes anemia and thrombocytopenia.[1,13] After chemotherapy, leukocyte count decreased, while Hb level and thrombocyte count increased. Evaluation of bone marrow aspiration after induction phase chemotherapy showed that all the patients were in remission, as determined by leukemic cell clearance from the bone marrow, mainly in the first 2 weeks after induction phase chemotherapy.[13]

Mean $CD4^+$, $CD8^+$ cells, and $CD4^+/CD8^+$ ratio were significantly decreased after induction phase chemotherapy (**Table 2**). Verma *et al.* reported a significant decrease in B cells, T cells, and NK cells approximately 2 weeks after chemotherapy.[14] Decreased $CD4^+/CD8^+$ ratio after chemotherapy in this study (P=0.004) was due to the larger decrease in $CD4^+$ cells than that in $CD8^+$ cells. Recovery of $CD4^+$ cells after chemotherapy was slower than recovery of $CD8^+$ cells. Chemotherapy decreases the $CD4^+$ and $CD8^+$ cells by increasing regulatory T cells (T reg), which supress the immune response by downregulating IL-2, and upregulating IL-10 and TGF-beta. Although IL-10 and TGF-beta are strong, immunosupressive factors, IL-2 is an important immune regulating factor. It is produced by T helper cells, which increase T cell proliferation, NK cell activity, and B cell antibody secretion. Past studies have shown that IL-2 concentration in ALL patients is low.[15,16]

After chemotherapy, apoptosis was not significantly decreased (P=0.689). In contrast, Laane *et al.* found an increase in apoptosis after chemotherapy.[17] Firstly, our results may have been caused by: 1) sampling time outside the window of maximal effect on apoptosis time (24 - 72 hours). Liu et al. reported a significant increase in apoptosis of lymphoblasts > 24 hours after induction phase chemotherapy in pediatric ALL patients, rather than in the early hours after chemotherapy.[18] Secondly, chemotherapy can cause necrosis, so lymphoblasts may not have been detected as apoptotic bodies.[19] Third, the subjects may have had chemotherapy drug resistance, although 100% of patients entered remission,[20] and forth, anti-apoptotic proteins levels (e.g., BCL-2, BCL-XL, etc.) in the patients were higher than pro-apoptotic proteins levels (e.g., BAX, BOK, BCL-Xs, BID, BAD, or Noxa).[18] Lymphoblast cells in ALL patients are more fragile than in healthy children, thus, apoptosis in ALL patients was not significantly higher than apoptosis in the healthy control group (**Table 3**).

There was a negative, significant correlation between apoptosis and $CD4^+$ and $CD8^+$ cells before chemotherapy (**Table 4**). This finding indicates that greater numbers of $CD4^+$ and $CD8^+$ cells result in decreased apoptosis. We noted that not only the number of $CD4^+$ and $CD8^+$ cells, but their function, was also important in immune response. The number and function of $CD4^+$ and $CD8^+$ cells before chemotherapy were correlated with subjects' response to chemotherapy and improved survival.[10]

Apoptosis had no significant correlation with $CD4^+/CD8^+$ ratio, either before or after chemotherapy. Similarly, Dewyer *et al.* suggested that $CD4^+/CD8^+$ ratio did not correlate with tumor response in induction phase chemotherapy.[21] No correlation between $CD4^+$ or $CD8^+$ cells and apoptosis after induction phase chemotherapy, may have been due to decreases of $CD4^+$, $CD8^+$ cells, and apoptosis after chemotherapy.

There was a significant correlation between $CD4^+$-delta (number of $CD4^+$ cells before chemotherapy minus the number of $CD4^+$ cells after chemotherapy) and apoptosis-delta (**Table 5**). We found that decreased $CD4^+$ cells lead to decreased apoptosis. The $CD4^+$ cells had no cytotoxic or cytolytic effect on tumor cells, but many cytokines produced by $CD4^+$ cells are needed for the development of $CD8^+$ into effector cells.[6-10]

The limitations of this study included the lack of an extended time to recognize the possibility of future relapse or resistance to chemotherapy drugs, apoptosis sample preparation was not accompanied by a specific marker for lymphocytes, so a series of monocytes may have been included, and the sampling time among patients after chemotherapy varied, potentially effecting the decreased apoptosis.

Comparing the values before and after induction phase of chemotherapy we conclude that: 1) $CD4^+$ and $CD8^+$ count cells not significantly higher, 2) there is no difference in apoptosis, 3) there was a negative correlation between apoptosis and $CD4^+$ and $CD8^+$ cells before induction phase chemotherapy in pediatric ALL patients, but no correlation after induction phase chemotherapy, 4) there is no correlation between $CD4^+/CD8^+$ ratio and apoptosis, 5) there is a significant correlation between $CD4^+$-delta and

apoptosis-delta, after induction phase chemotherapy. $CD4^+$ and $CD8^+$ cells, and $CD4^+/CD8^+$ ratio can be used to predict apoptosis before chemotherapy, while $CD4^+$-delta may be useful to predict apoptosis after induction phase response to chemotherapy in ALL patients.

Suggestions for future research also include determining the best time of sampling after chemotherapy (24 hours, 36 hours, 48 hours, or 72 hours) to obtain results with significantly increased apoptosis, compared to what our study showed, and to assess the profile of the percentage of T reg and expression of cytokines in ALL patients before and after chemotherapy.

Conflict of Interest

None declared.

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Original Article

Serum ferritin, serum nitric oxide, and cognitive function in pediatric thalassemia major

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Abstract

Background Hemolysis and repeated blood transfusions in children with thalassemia major cause iron overload in various organs, including the brain, and may lead to neurodegeneration. Hemolysis also causes decreased levels of nitric oxide, which serves as a volume transmitter and slow dynamic modulation, leading to cognitive impairment.

Objective To assess for correlations between serum ferritin as well as nitric oxide levels and cognitive function in children with thalassemia major.

Methods This analytical study with cross-sectional design on 40 hemosiderotic thalassemia major patients aged 6-14 years, was done at the Thalassemia Clinic in Dr. Hasan Sadikin Hospital, Bandung, West Java, from May to June 2015. Serum ferritin measurements were performed by an electrochemiluminescence immunoassay; serum nitric oxide was assayed by a colorimetric procedure based on Griess reaction; and cognitive function was assessed by the Wechsler Intelligence Scale for Children test. Statistical analysis was done using Spearman's Rank correlation, with a significance value of 0.05.

Results Abnormal values in verbal, performance, and full scale IQ were found in 35%, 57.5% and 57.5%, respectively. Serum nitric oxide level was significantly correlated with performance IQ (P=0.022), but not with verbal IQ (P=0.359) or full scale IQ (P=0.164). There were also no significant correlations between serum ferritin level and full scale, verbal, or performance IQ (P=0.377, 0.460, and 0.822, respectively).

Conclusion Lower serum nitric oxide level is significantly correlated to lower cognitive function, specifically in the performance IQ category. However, serum ferritin level has no clear correlation with cognitive function. **[Paediatr Indones. 2017;57:148-52 doi: http://dx.doi.org/10.14238/pi57.3.2017.148-52]**.

Keywords: thalassemia major; ferritin; nitric oxide; cognitive function; WISC-R

Thalassemia major is the most severe form of a group of inherited hemoglobin disorders, due to the partial or total absence of hemoglobin.[1, 2] The use of intense therapy increases life expectancy as well as the frequency of complications. Children with thalassemia major have multiple risk factors for developing central nervous system (CNS) complications. Hemolysis and repeated blood transfusions cause decreases in nitric oxide levels and iron overload in various organs, including the brain. Increased iron in the brain leads to oxidative stress and possible irreparable brain tissue damage, causing cognitive impairment.[3-5] Ferritin is a protein that stores iron and exists in all tissues including the brain. Serum ferritin level is a good marker for assessing body iron stores. Nitric oxide (NO), a diffusible intercellular messenger, is produced by most mammalian cells, including neurons.[6] Nitric oxide seems to be involved in several aspects of cognition, among which learning and memory are

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implicated, as nitric oxide can act as a retrograde messenger during long term potentiation.[7] Nitric oxide also serves as a volume transmitter and slow dynamic modulator. As such, the decreased level of NO in children with thalassemia major may lead to cognitive impairment.[8]

In most cases, neurological involvement in thalassemia major does not initially present with relevant signs and symptoms (subclinical), and can only be detected during neurophysiological and neuropsychological evaluation.[9] Neuropsychological tests are safe and reliable for diagnosis of cognitive impairment in ß-thalassemia major patients, and they may even facilitate early diagnosis.[10] The *Wechsler Intelligence Scale for Children* (3rd edition) is the most widely used intelligence test for school aged children and adolescents.[11] Early diagnosis through regular neuropsychological testing and appropriate treatment of CNS complications are essential to improve the quality of life of children with thalassemia major.[10] We aimed to evaluate possible correlations between serum ferritin and nitric oxide levels with cognitive function in children with thalassemia major.

Methods

This cross-sectional study was performed in the Thalassemia Outpatient Clinic at the Department of Child Health, Dr. Hasan Sadikin Hospital, Bandung, West Java, from May to June 2015. Subjects were selected by consecutive sampling of hemosiderotic thalassemia major pediatric patients. The inclusion criteria comprised of children diagnosed with thalassemia major aged between 6-14 years with regular blood transfusions and iron chelation treatment, normal body mass index, formal education, and no fever. The exclusion criteria were as follows: (a) had a history of major mental disorders with delayed milestone development; (b) had physical disabilities that could interfere with performance, such as deafness or blindness; (c) received prior treatment with drugs known to be neurotoxic; or (d) had a history of chronic medical illness other than thalassemia that could affect cognition.

Based on the calculated required sample size, our study included 40 subjects with hemosiderotic thalassemia major. Informed consent was obtained from subjects' parents and the study was approved by the Ethics Committee of the Universitas Padjadjaran Medical School/Dr. Hasan Sadikin General Hospital. Data on adherence to iron chelating agent use, frequency of transfusion, and school attendance were collected from medical records and questionnaires.

The eight parameters of the hematology test were based on flow cytometry. The serum ferritin quantitative test, an electrochemiluminescent immunoassay (ECLIA), was done at the Clinical Pathology Laboratory of the Hasan Sadikin Hospital, Bandung.[12] Serum nitric oxide was assayed using a colorimetric procedure based on Griess reactions at the *Prodia Laboratory Centre*, Jakarta.[13] Cognitive function was assessed with the Indonesian version of the *Wechsler Intelligence Scale for Children-Revised* (WISC-R), which provided outputs of their verbal and performance subtests and a combined full scale IQ test. The evaluation was performed by a qualified clinical child psychologist at Hasan Sadikin Hospital, Bandung. Patients' blood was drawn just before blood transfusion and the WISC-R test was performed after blood transfusion. Study results were analyzed by *SPSS version 20.0* software. Numeric data are presented as means, standard deviations, and medians with range. The correlations between serum ferritin and serum nitric oxide with cognitive function were determined with Spearman's Rho coefficient correlation. Results with P values <0.05 were considered to be statistically significant.

Results

Forty thalassemia major patients were included in this study. The mean age of patients (18 males and 22 females) was 10.03 (SD 1.94) years. The median age of transfusion onset was 7 (range 3-24) months. The mean duration of illness was 102.48 (SD 27.99) months. The characteristics of subjects are summarized in **Table 1**. The median ferritin level was 3,710 (range 1,043−11,200) μg/L, and the mean nitric oxide level was 21.83 (SD 9.7) μM. Other hematological parameters are shown in **Table 2**.

Table 1. Characteristics of subjects

Characteristics	N=40
Gender, n(%)	
Male	18 (45)
Female	22 (55)
Mean age (SD), years	10.03 (1.94)
Median age of onset (range), months	7 (3-24)
Median onset of blood transfusion (range), years	7.5 (3-24)
Median onset of chelation therapy (range), years	36 (12-96)
Mean duration of illness (SD), months	102.48 (27.99)
Frequency of blood transfusion per year, n(%)	
< 15	5 (12)
15-20	23 (58)
20-25	8 (20)
25-30	4 (10)
Chelation type, n(%)	
Desferrioxamine	2 (5)
Deferasirox	11 (28)
Deferiprone	27 (67)
Compliance to chelation, n(%)	
Optimal	30 (75)
Not optimal	10 (25)
School attendance per month, n(%)	
50-75%	4 (10)
75-100%	36 (90)
Education, n(%)	
Elementary school	37 (93)
Junior high school	3 (7)
Mean years of schooling (SD), years	3.85 (1.87)

The abnormal values of verbal IQ, performance IQ, and full scale IQ were 35%, 57.5%, and 57.5%, respectively, as shown in **Table 3**. Serum nitric oxide level was significantly correlated with performance IQ (P=0.022) (**Table 4**), but not with verbal IQ (P=0.359) or full scale IQ (P=0.164). There were no significant correlations between serum ferritin level and full scale IQ (P=0.377), verbal IQ (P=0.460), or performance IQ (P=0.822), as also shown in **Table 4**.

Discussion

Of our 40 subjects, 35% had abnormal verbal IQ, 57.5% had abnormal performance IQ, and 57.5% had abnormal full scale IQ (**Table 3**). These abnormal results were higher than reported by Economou *et al.*, who found that 36.4% of patients with ß-thalassemia major had abnormal full scale IQ.[14] In contrast, we also found two subjects with a high average full scale IQ of 117. Factors underlying the high IQ scores in two subjects were likely due to the subjects' young age (6 years), their good nutritional status, short disease duration (5 years), and relatively high hemoglobin levels compared to the other subjects. Similarly, Raafat et al. reported that 4 (0.04%) patients with ß-thalassemia major had superior IQ scores and 20 (0.2%) patients had high average IQ scores.[15]

We observed a significant correlation between serum nitric oxide levels and performance IQ, but no significant correlation with verbal or full scale IQ (**Table 4**). The pathology of thalassemia disease includes accelerated destruction of nitric oxide and limited compensation processes to increase nitric oxide production. Hence, the deficiency of nitric oxide causes a decrease in cognitive function in children with thalassemia major.[16] The significant correlation between low serum nitric oxide level and abnormal performance IQ might be due to low hemoglobin and low nitric oxide levels caused by hemolysis during the critical stages of early childhood development, affecting parts of the brain associated with performance IQ. Children with thalassemia major have severe anemia at an early age, i.e., before the age of 2 years, which can affect the development of performance IQ. On the other hand, hypoxia and chronic anemia tend to decrease performance

Table 2. Biochemical parameters of subjects

		Reference values
Median hemoglobin (range), g/dL	7.2 (4.1–9.5)	11.5–15.5
Median hematocrit (range), %	21 (12–28)	35–45
Median MCV (range), fl	75.1 (62.4–84.2)	77–95
Median MCH (range), pg	25.8 (20–28.2)	25–33
Median MCHC (range), %	33.9 (31.3–35.5)	31–37
Median erythrocyte (range), x106 microliter	2.85 (1.5–4.0)	4.43–6.02
Median leukocyte (range), /mm3	5,500 (4,000–8,800)	4,500–13,500
Median serum ferritin (range), ng/mL	3,687.5 (1,043–11,200)	14–124
Mean serum nitric oxide (SD), µM	21.83 (9.7)	50.6–121.26

Table 3. The Wechsler Intelligence Scale test interpretations

Variables	n						
	n(%)	Superior	HA	Average	LA	Borderline	MD
Verbal IQ		1	1	24	10	4	-
Normal	26 (65)						
Abnormal	14 (35)						
Performance IQ		1	1	15	18	5	-
Normal	17 (42.5)						
Abnormal	23 (57.5)						
Full scale IQ		-	2	15	18	5	-
Normal	17 (42.5)						
Abnormal	23 (57.5)						

HA=high average, LA=low average, MD=mental deficits

Table 4. Correlation between serum ferritin and serum nitric oxide levels with cognitive function parameters of the WISC test

Variables	Verbal IQ		Performance IQ		Full scale IQ		Interpretation	
	r	P value	r	P value	r	P value	r	P value
Serum ferritin	-1.20	0.460	-0.037	-0.822	-0.144	0.377	-0.080	0.626
Serum nitrit oxide	0.149	0.359	0.360	0.022	0.225	0.164	0.240	0.136

capability to lower than verbal ability, since tests for performance IQ require a larger energy supply. In accordance with this theory, a longitudinal cohort study by Ai *et al.* in 171 children in China showed that children with low hemoglobin levels had a lower performance IQ, but normal verbal IQ.[17] Nitric oxide controls the transmission of information and plays a role in cognitive function, such as in learning and memory, so that NO deficiency may cause cognitive impairment. We found no correlation between verbal IQ or full scale IQ and serum NO levels. Low nitric oxide levels in thalassemia may not influence verbal IQ and full scale IQ, since the verbal IQ test assesses general knowledge, comprehension, and fluency in speaking. To date, no other study has been performed on the possible correlations between serum nitric oxide levels and verbal, performance, and full scale IQs.

We found no correlation between serum ferritin levels and cognitive function in children with thalassemia major, in either full scale, verbal, or performance IQs (**Table 4**). Excess iron causes elevated levels of serum transferrin and iron transport to pass through the blood-brain barrier, causing the accumulation of iron in the brain.[18] Furthermore, the iron can catalyze the formation of free radicals that cause oxidative stress which will accelerate brain tissue degeneration that might lead to cognitive impairment.[5] Our results showed no significant correlation between serum ferritin levels and cognitive function, possibly due to the lack of signs of hemosiderosis in the brain caused by iron overload. A previous study reported that ß-thalassemia major subjects aged >16 years with signs of systemic hemosiderosis had significantly lower neuropsychological test scores.[19] In addition, serum ferritin levels also have low specificity as a marker of hemosiderosis because the levels are very susceptible to infections and inflammatory conditions.[20] The results of this study were similar to those of Monastero *et al.*, which showed no significant association between ferritin levels and cognitive function in ß-thalassemia major.[19] Similarly, Raafat *et al.* showed no correlation between serum ferritin level and full-scale IQ, verbal IQ, or performance IQ.[15] However, Retnani found a correlation between serum ferritin level and performance IQ, but no significant correlation between serum ferritin level and verbal or full scale IQ.[21]

The limitations of this study were the lack of comparative assessment of serum nitric oxide levels in healthy subjects, and that the high levels of serum ferritin cannot be used to evaluate iron deposition in tissue, especially in the brain. A more accurate test is needed to assess the level of iron deposition in the

brain.

In conclusion, lower serum nitric oxide level is significantly correlated with lower cognitive function, specifically in performance IQ. However, serum ferritin levels have no clear correlations with cognitive function.

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Conflict of interest

None declared.

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Original Article

Behavioral parent training for ADHD children: a mixed methods study

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Abstract

Background Management of ADHD requires multimodal treatments. Parental participation is one of the most important factors for effective ADHD treatment.

Objective To investigate the effectiveness of behavioral parent training combined with routine clinical care, in reducing ADHD symptoms in children.

Methods Quantitative and qualitative methods were combined in this study. This study was conducted at 3 growth and developmental clinics in Central of Java, on June-July 2016. The quantitative aspect was assessed by comparing ADHD quotient scores at pre- and post-intervention, while the qualitative aspect by intensive parental interviews. Parents of children with ADHD were randomized with block random sampling. In the treatment group, parents received behavioral training for 7 weeks, along with weekly routine clinical care for their children. The control group received only routine clinical care of the children. Six parents in the treatment group were randomly selected for intensive interviews.

Results A total of 67 parents with their children were involved. Both groups' ADHD quotient scores improved post-intervention. The treatment group ADHD quotient score was reduced from 120.53 to 116.41 (effect size Cohen's *d* 0.68). The control group ADHD quotient score was reduced from 121.74 to 119.83 (effect size Cohen's *d* 0.23). Mean difference post-intervention in both group was not significant (p=.161). After behavioral parent training, communication between parents and children increased and parents' capability in directing their children's daily activity increased.

Conclusion Behavioral parent training can not enhacing effectiveness of routine clinical care to reduce ADHD symptoms in children.

Keywords: *attention deficit/hyperactivity disorder; behavioral parent training*

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Attention deficit/hyperactivity disorder (ADHD) is the most common neurobehaviour disorder in children, requiring specific treatments. If not properly treated, ADHD may persist through adolescence. Attention deficit/hyperactivity disorder causes functional disorders, such as difficulty in academic achievement or social interaction,

adolescent mischievousness, and increased risk of traffic accidents.[1-3] Worldwide, the prevalence of ADHD varies from 4% to 57%, with average about 12%, depending on the instruments used for screening or diagnosis. The male and female ratio was reported to be 4:1.[4] Using the *Skala Penilaian Perilaku Anak Hiperaktif Indonesia* (SPPAHI) for screening, the ADHD incidence in Jakarta was 26.2%.[5] A Yogyakarta study using the same screening test, showed an ADHD incidence of 21-24%.[6]

Children with ADHD need multimodal treatments. Parental participation is one of the most important factors for effective ADHD treatment.[2,3,7] Based on our observations, the common obstacles to effective ADHD treatment are: (1) lack of knowledge - pediatricians, occupational therapists, and parents do not have enough knowledge about ADHD; (2) lack of time - lack of adequate time for parents to consult a doctor; (3) lack of funds - many parents with ADHD children do not have insurance or enough funds to cover the cost of the ADHD treatment programs; (4) lack of parental involvement in the treatment of their child.

The Agency for Health Care Research and Quality (AHRQ) reviewed the comprehensive treatment of ADHD in preschool-aged children and suggested that behavioral parent training had good efficacy in reducing ADHD symptoms in children of this age group.[7] The objective of this study was –to investigate the effectiveness of behavioral parent training combined with routine clinical care in reducing ADHD symptoms in children and also to investigate the response from the parents who received training.

Methods

This mixed methods study combined quantitative and qualitative methods. The quantitative aspect was assessed by comparing ADHD quotient scores pre- and post-intervention. The qualitative aspect was assessed by intensive parent interviews. The study was conducted at the Growth and Developmental Clinic of Dr. Zainudin Arif Mental Hospital, Solo, the Growth and Developmental Clinic of Dr. Soejarwadi Mental Hospital, Klaten, and the Rehabilitation Clinic of Dr. Sardjito General Hospital, Yogyakarta, from June to July 2016. Subjects were parents of ADHD children aged 3 to 7 years who were diagnosed with ADHD, based on the diagnostic and statistical manual of mental disorders (DSM) V. Parents who had consulted psychologists or whose children were on ADHD medication were excluded from this study.

The ADHD test by James E. Gilliam was used to measure ADHD quotient.[8] The ADHD test was done independently by a permitted and licensed practitioner, who was blinded to the identity of the parent groups. Parents were randomized with block random sampling according to the site, in order to determine group placement. In the treatment group, parents received behavioral training once per week for 7 weeks (**Table 1**) and weekly routine clinical care for their children. In the control group, children received only the weekly routine clinical care. Behavioral parent training was conducted by the researchers, and was comprised of different training modules each week (**Table 1**). After the intervention, children in both groups underwent repeat testing to measure their ADHD quotient scores. Intensive interviews were performed on 6 parents in the treatment group, comprising two parents from each site who were randomly selected. This study was approved by the Ethics Committee of Gadjah Mada University Medical School.

Table 1. Behavioral parent training modules

Week	Topics
I	What is ADHD?
II	Improving communication
III	Behavior problem or sensory problem?
IV	Understanding the behavior problem and behavioral modification
V	Understanding the sensory problem and play modification
VI	Independent routine activities
VII	Material review

Results

A total of 67 parents with children who had been diagnosed with ADHD participated in this study. Four parents were excluded, because they had received counseling on behavior modification by psychologists (3), or

refused to join the study (1). A total of 19 subjects came from Dr. Zainudin Arif Mental Hospital, Solo (9 subjects in the treatment group); 24 subjects came from RSJD Dr. Soedjarwadi Mental Hospital, Klaten (12 subjects in the treatment group); and 24 subjects came from Dr. Sardjito Hospital, Yogyakarta (12 subjects in the treatment group). Subjects' baseline characteristics were similar between groups (**Table 2**).

Table 2. Subjects' characteristics

	Treatment group (n=33)	Control group (n=34)
Male sex, n	29	30
Mean age, months	59.6	61.9
Maternal education, n		
Junior high school	7	6
Senior high school	26	28
Paternal education, n		
Junior high school	3	3
Senior high school	30	31
Monthly income, n		
< IDR 1 million	6	5
IDR 1-2 million	25	26
> IDR 2 million	2	3

In the treatment group, the mean ADHD quotient scores were 120.53 pre-intervention and 116.41 post-intervention (effect size Cohen's d=0.68). In the control group, the mean ADHD quotient scores were 121.74 pre-intervention and 119.83 post-intervention (effect size Cohen's d=0.23) (**Table 3**). Mean difference of ADHD quotient scores post-intervention was not significant (P=0.161).

Table 3. Pre- and post-intervention ADHD quotient score

	Pre	Post	Effect size Cohen's *d*
Treatment group	120.53	116.41	0.68
Control group	121.74	119.83	

Six parents in the treatment group (2 parents from each site) underwent intensive interviews. After behavioral parent training, 3 of 6 parents succeeded in giving clear instructions for daily activities, improving communication with their children, and forbidding them to watch television. Two of 6 parents recognized reduced symptoms of hyperactivity in their children (**Table 4**).

Table 4. Intensive parental interviews (6 parents)

Benefit	n
Giving clear instructions for daily activities	3
Increased communication skill	3
forbidding their child to watch television	3
Decreased hyperactivity	2
Calmer, not cranky	1
Child completed given tasks	1
Maintained a routine of activities	1
Child participated in sports	1

Discussion

We conducted a combination of qualitative and quantitative methods, in order to assess the effectiveness of behavioral parent training combined with routine pediatric clinical care. Subjects in this study were parents with children aged 3 to 7 years who had been diagnosed with ADHD. The children had not received pharmacological treatment and parents had not received education on behavioral disorders. This was done to avoid pharmacological and educational bias effects on the ADHD quotient scores.

The main result of this study was a decrease in ADHD quotient score from 120.53 at pre-intervention to 116.41 at post-intervention, in the treatment group. But this decline in the ADHD quotient score was not clinically significant, as these scores were in the in the same level of severity category. Van den Hoofdakker *et al.* found that behavioral parent training combined with routine clinical care of children showed better results compared to routine clinical care alone.[9] Our quantitative method was intensive parental interviews. These interviews revealed that the

most common benefits of behavioral parent training were in giving clear instructions for daily activities and increased communication between parents and children. Other studies also showed that behavioral parent training was effective in reducing ADHD symptoms compared to children with ADHD on the waiting list for routine clinical care.[10,11]

Some limitations of this study were (1) lack of monitoring parental compliance in implementing the behavior modification program at home, as this issue could not be routinely monitored or clearly documented; (2) researchers could not prevent communication between parents in the study. As such, parents in control group may have also implemented the behavior training at home by using information from parents in the treatment group; (3) the time allocated to evaluate changes in ADHD quotient scores was very short.

In conclusion, behavioral parent training is not enhacing effectiveness of routine clinical care to reduce ADHD symptoms in children.

Conflict of Interest

None declared.

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Original Article

Efficacy of oral erythromycin to enhance feeding tolerance in preterm infants

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Abstract

Background Feeding intolerance is a common condition that affects preterm infants. Erythromycin is a prokinetic agent used to treat feeding intolerance, but its efficacy remains inconclusive.

Objective To evaluate the effectiveness of oral erythromycin to enhance feeding tolerance in preterm infants.

Methods This prospective, randomized controlled trial in preterm infants was conducted at Sanglah Hospital, Denpasar, Bali, from June 2015 to January 2016. Eligible infants were randomized to receive either 12.5 mg/kg/dose oral erythromycin or a placebo, every 8 hours. The primary outcome was the time to establish full enteral feeding. The secondary outcomes were body weight at full enteral feeding and length of hospital stay.

Results Of 62 initial subjects, 3 infants dropped out of the study. Thirty infants were given erythromycin and 29 infants were given placebo. The baseline characteristics of the two groups were similar, with mean of gestational ages of 31.4 (SD 1.7) weeks in the erythromycin group and 32.4 (SD 2.2) weeks in the placebo group. The median times to reach full enteral feeding did not significantly differ between the two groups, with 10 (SD 5.3) days in the erythromycin group *vs.* 8 (SD 6.5) days in the placebo group (P=0.345). Also, median body weights at full enteral feeding and lengths of hospital stay were not significantly different between the two groups.

Conclusion Erythromycin of 12.5 mg/kg/dose every 8 hours as prophylactic treatment does not significantly enhance feeding tolerance in preterm infants. Median body weights at full enteral feeding and length of hospital stay are not significantly different between the erythromycin and placebo groups. **[Paediatr Indones. 2017;57:154-9 doi: http://dx.doi.org/10.14238/pi57.3.2017.154-9]**.

Keywords: *erythromycin; feeding intolerance; preterm infants*

Feeding intolerance is a common problem in managing preterm infants. Feeding intolerance presents as gastric residual, regurgitation, recurrent vomiting, or abdominal distention, in severe cases. Feeding intolerance leads to poor weight gain, longer hospital stay, and potential hospital-acquired infection, due to central inserted catheter (umbilical catheter, central venous catheter, percutaneous inserted central catheter), and long-term parenteral nutrition. The most common cause of feeding intolerance is low gut motility due to prematurity.[1,2]

Prokinetics are commonly used to treat feeding intolerance in preterm infants. The most widely used prokinetics are metoclopramide, cisapride, and domperidone. Some serious adverse effects have been related to these prokinetics, such extrapyramidal reactions and lethargy with metoclopramide and QT interval widening with cisapride. In addition, the use of domperidone in preterm infants remains controversial.[3,4]

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Erythromycin is an antibiotic also commonly used to treat feeding intolerance in preterm infants. This macrolide has a motilin-like effect and stimulates peristalsis.[1,5] Erythromycin works as a motiline agent, by binding to the motiline receptor at the antrum and upper duodenum and leading to increased contractions in the antrum.[4,6] The motiline hormone stimulates the gastric emptying process and induces phase III of migrating motor complexes (MMC) in the proximal intestine, reducing transit time in the intestine.[7,8] This process is not achieved until 32 weeks of gestational age.[9,10] Some studies in infants with intestinal dismotility showed benefits from erythromycin,[9,11,12] while others gave inconsistent results.[13,14] We aimed to assess the efficacy of high-dose erythromycin (12.5 mg/kg) as prophylactic management to enhance feeding tolerance in preterm infants, compared to a placebo.

Methods

This randomized controlled trial was done in preterm infants at 28 to <37 weeks gestational age and admitted to the Neonatology Ward, Sanglah Hospital, Denpasar, Bali, from June 2015 to January 2016. Subjects were randomized into two groups: one group was given a high dose of erythromycin (12.5 mg/kg t.i.d.) and the other group was given a placebo).

The sample size was calculated to be 25 per group, for 5% significance level (α) and 80% power (ß), based on ORs from a previous study.[7] Using a 10% estimate of lost-to-follow up, the minimum required sample size was calculated to be 60 subjects.

Study subjects were recruited by consecutive sampling until the minimum sample size was achieved. Exclusion criteria were: children with intestinal bleeding, necrotizing enterocolitis, major congenital anomaly, previous history of abdominal surgery, or referred to another center. Block randomization was performed using computer software (*SPSS 20.0 for Mac*), sealed, and kept in the Pharmacy Division until the study ended.

The treatment group was given high-dose, oral, erythromycin (12.5 mg/kg) every 8 hours while the control group was given a placebo. Erythromycin syrup and placebo syrup were prepared by the Pharmacy Division of Sanglah Hospital. Both preparations were similar in color and labeling. The investigators, clinicians, nurses, and parents were blinded to the contents during the study. Intervention was started from the initial feeding and continued until full enteral feeding was achieved. Complications such sepsis and necrotizing enterocolitis, as well as secondary outcomes of body weight when the full enteral feeding achieved, and length of stay were noted. Any adverse effects such as diarrhea, arrhythmia, and pyloric stenosis hypertrophy were noted in all subjects. Infants with serious adverse events were dropped from the study, and treated accordingly. This study was approved by the Ethics Committee of Sanglah Hospital, Denpasar. Subjects' parents provided written informed consent.

Characteristics of subjects, adverse events, and data on full enteral feeding were collected and shown in tables. Associations of time taken for full enteral feeding, body weight when full enteral feeding was achieved, length of hospital stay, and the intervention were analyzed using Mann-Whitney U test, due to non-normal distribution of data. Analyses were performed with *SPSS 16.0* software.

Results

A total of 62 subjects enrolled in the study. Three subjects dropped out: two infants in the treatment group due to worsening of condition or incomplete data during analysis, and one subject in the placebo group due to worsening condition. The remaining 59 subjects were analyzed, 30 subjects in the treatment group and 29 subjects in the control group, as seen in the flow chart in **Figure 1**.

Subjects' characteristics were similar between groups (**Table 1**). There were more males in the treatment group than in the placebo group. Modes of delivery were similar in both groups. Gestational age was younger in the treatment group than in the placebo group [31.4 (SD 1.7) *vs.* 32.4 (SD 2.2) weeks, respectively]. Mean birth weight was similar in both groups (about 1,550 grams). The placebo group had 3 small-for-gestational age infants and the treatment group had none. Severe asphyxia was higher in the treatment group (9/30) compared to the placebo group (2/29). Ventilator support was higher in the treatment group (10%) compared to the placebo group (6.9%).

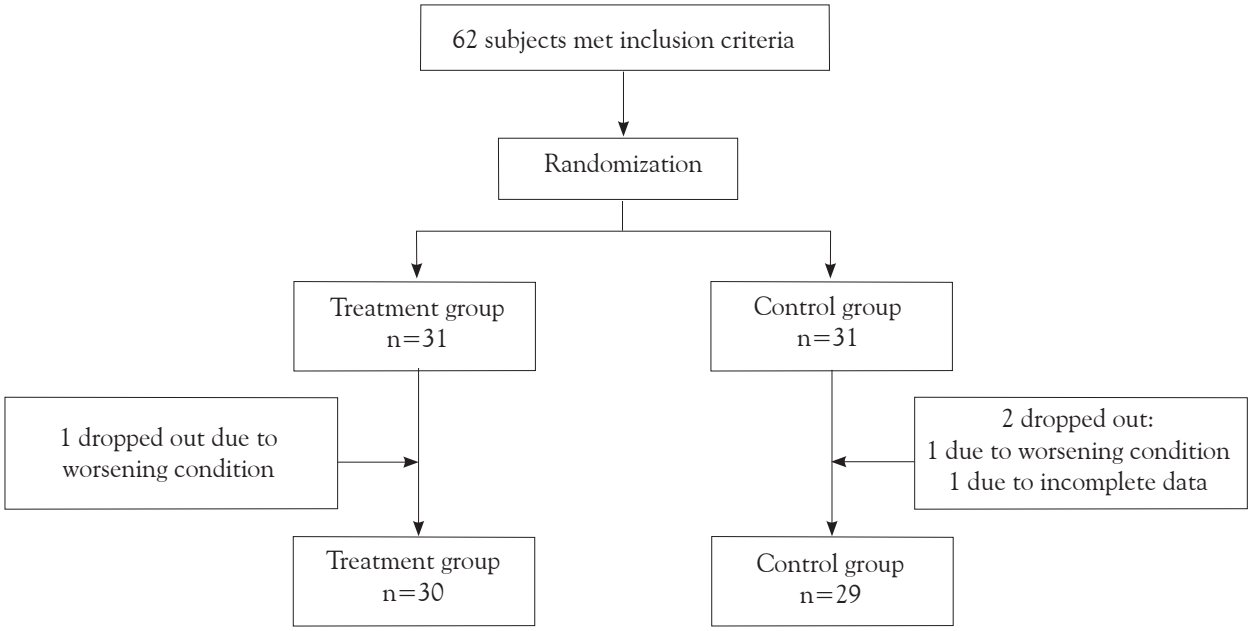

Figure 1. Study flow chart

Table 1. Subjects' characteristics

Characteristics	Group	
	Treatment (n=30)	Placebo (n=29)
Male gender, n	18	13
Mean birth weight (SD), grams	1,549 (270)	1,543 (288)
Intrauterine growth parameter		
Appropriate-for-gestational age	30	26
Small-for-gestational age	0	3
Mean gestational age (SD), weeks	31.4 (1.7)	32.4 (2.2)
Delivery mode, n		
Vaginal	14	15
Cesarian section	16	14
Severe asphyxia, n		
Yes	9	2
No	21	27
Oxygen support, n		
No oxygen	4	4
CPAP	23	19
High-flow nasal cannula	0	4
Ventilator	3	2
Nutritional support, n		
Breast milk	15	13
Formula	3	4
Breast milk and formula	12	12

Continuous positive airway pressure (CPAP) support was also higher in the treatment group (76.7%) compared to the placebo group (65.5%).

Similar characteristics of sepsis, initial time for trophic feeding, feeding intolerance, diarrhea, hypertrophic pyloric stenosis, and arrhythmia were found in both groups, as shown in **Table 2**.

Median full enteral feeding was achieved faster in the placebo group than in the treatment group, but the body weight when full enteral feeding was achieved

Table 2. Complications and adverse events

Complications	Group		P value*
	Treatment (n=30)	Placebo (n=29)	
Sepsis (clinical, culture), n	16	20	0.288
Mean initial time for trophic feeding (SD), days	4.6 (3.1)	6.0 (6.6)	0.286
Vomiting, n	4	2	0.671
Gastric residual, n	7	5	0.748
Diarrhea, n	0	0	0
Hypertrophic pyloric stenosis, n	0	0	0
Arrythmia, n	0	0	0
Cholestasis, n	1	7	0.026

*Chi-square tests

was higher in the treatment group. Length of hospital stay was longer in the treatment group compared to the placebo group. However, none of these differences were statistically significant, as shown in **Table 3**.

Subjects given formula achieved full enteral feeding faster than subjects given breast milk exclusively or a combination of breast milk and formula in both the treatment and placebo groups. Subjects with gestational age >32 weeks also achieved full enteral feeding faster compared to subjects with lower gestational age, in both groups. Subjects with severe asphyxia took longer to achieve full enteral feeding compared to subjects without severe asphyxia, in both groups, as shown in **Table 4**.

Discussion

Past studies have evaluated the efficacy of erythromycin as a prokinetic in preterm infants, be it therapy or prophylaxis. In this study, high-dose erythromycin was given as prophylaxis for feeding intolerance to preterm infants with gestational age less than 37 weeks. No sub-group analysis for gestational age was made due to the large sample size and long period of study required for such analyses.

Baseline characteristics of subjects were similar between groups. Sub-group analysis for enteral feeding revealed that subjects given formula achieved full enteral feeding faster than subjects given breast milk. This result may have been due to lack of available breast milk in the first few days after admission, since our hospital does not have breast milk bank. As such, the initial time for trophic feeding in breast milk subjects may have been delayed.

Full enteral feeding was achieved faster in the

Table 3. Analysis of time taken to full enteral feeding, body weight at full enteral feeding, hospital length of stay, and interventions

Complications	Group		P value*
	Treatment (n=30)	Placebo (n=29)	
Time taken until full enteral feeding, day			
Median (interquartile range)	10 (5.3)	8 (6.5)	0.345
Minimum – maximum	2-34	2-39	
Median body weight at full enteral feeding, grams (interquartile range)	1,600 (283.0)	1,540 (433)	0.305
Length of hospital stay, days			
Median (interquartile range)	25.5 (24.0)	24.0 (20)	0.710
Minimum – maximum	4-68	9-8.6	

Table 4. Time to achieve full enteral feeding according to gestational age, enteral nutrition, and history of severe asphyxia

Variables	Mean time to achieve full enteral feeding (SD), days	
	Treatment (n=30)	Placebo (n=29)
Median enteral nutrition		
Breast milk	9.0 (4.9)	10.0 (6.1)
Formula	6.0 (7.3)	8.0 (10.8)
Breast milk + formula	11.0 (9.0)	10.0 (8.4)
Median gestational age		
≤ 32 weeks	11.0 (9.6)	11.0 (8.2)
> 32 weeks	8.0 (6.1)	9.0 (6.5)
Severe asphyxia		
Yes	13.0 (9.6)	13.0 (10.5)
No	8.0 (7.0)	9.0 (4.2)

placebo group than the treatment group (8 *vs.* 10 days, respectively), but this result was not significant. Studies with low-dose erythromycin as a prokinetic have had inconsistent results. Previous studies found that low-dose erythromycin gave no prophylactic benefit in infants born at <32 weeks with feeding intolerance.[9,11] In contrast, Oei *et al*. found that low-dose erythromycin (2.5 mg/kg q.i.d) was beneficial as a prophylactic against feeding intolerance,[14] with significantly shorter time to reach full enteral feeding in the treatment group than in the placebo group [6.0 (SD 2.3) *vs.* 7.9 (SD 3.5) days, respectively]. This difference may have been due to different sample sizes and methods among studies.

Inconsistent results were found in some studies about the use of erythromycin as a rescue protocol in feeding intolerance in preterm infants. Most studies have shown a benefit with high-dose erythromycin (>10 mg/kg t.i.d.),[12,15,16] but no significant benefit with low-dose erythromycin, in management of feeding intolerance.[7,17,18] One study found a significant benefit with high-dose erythromycin (12.5 mg/kg q.i.d) compared to placebo for treating feeding intolerance in preterm infants with birth weight <1,500 grams [13.5 (8-22) *vs*. 25 (16-33) days, respectively].[5] Also, Madani et al. reported that a significant benefit was found in preterm infants >32 weeks who took high-dose erythromycin (12.5 mg/kg q.i.d) compared to placebo.[2] Similarly, Aly et al. found a significant benefit in preterm infants >32 weeks [10.5 (4.1) *vs.* 16.3 (5.7) days, respectively, with low-dose erythromycin (1 mg/kg t.i.d) compared to placebo.[1] Furthermore, a Jakarta study found no significant difference in the use of low-dose erythromycin (3 mg/kg q.i.d.) in preterm infants compared to placebo.[4]

In this study, we found no significant difference in the use of high-dose erythromycin as prophylaxis for feeding intolerance in preterm infants <37 weeks, compared to placebo. Two types of motilin receptors (neural and smooth muscle) have been found and the efficacy of erythromycin depends on the dose and gestational age.[6,12] Low-dose erythromycin (1-3 mg/kg) can stimulate neural motilin receptor (cholinergic nerves of the gut at both preganglionic and postganglionic levels) and induce phase III of MMC. But the motilin receptor in smooth muscle can only be stimulated by high-dose erythromycin.[6,12,13] The MMC is immature before 32 weeks of gestation. Higher dose erythromycin is needed in preterm infants <32 weeks gestational age to stimulate antrum contraction and antro-duodenal coordination.[11,13]

We found no benefit of high-dose erythromycin for preventing feeding intolerance in preterm infants. Our findings may have been due to insufficient intestinal dysmotility in our subjects to show the effects of erythromycin. Sub-group analysis showed that preterm infants >32 weeks reached full enteral feeding faster compared to those with gestational age <32 weeks, in both the treatment and placebo groups. We also found that preterm infants in the treatment group had higher body weight at full enteral feeding compared to the placebo group [1,600 (SD 249) *vs.* 1,540 (SD 269) grams, respectively], but the treatment group also had a longer length of stay [25.5 (SD 24.0) *vs.* 24 (SD 20.0) days, respectively). This difference may have been due to the younger gestational age of subjects in the treatment group.

Complications of parenteral nutrition are sepsis and cholestasis. In our study, sepsis and cholestasis were higher in the placebo group. Nevertheless, the placebo group took a shorter time to achieve full enteral feeding, indicating that the sepsis and cholestasis were not related to parenteral feeding. With regards to safety of erythromycin, we found no diarrhea, arrhythmia, or hypertrophic pyloric stenosis in the treatment group, similar to other studies.[16,19,20]

Long-term erythromycin can change the normal intestinal flora, with high-dose or therapeutic doses leading to diarrhea and sepsis.[21,22] However, Ng found no intestinal microorganism changes after the use of erythromycin for 10 days and 4 weeks. He also found no infection or necrotizing enterocolitis outbreaks during the 69-month study.[12] We did not examine our subjects' intestinal microorganisms before or after erythromycin.

In conclusion, no significant differences in the time to reach full enteral feeding, Body weight when full enteral feeding is achieved, or length of stay are observed in the use of high-dose erythromycin as a prophylactic for feeding intolerance in preterm infants compared to placebo. Further study with sub-groups of various gestational ages can give us a better understanding of the efficacy of erythromycin as a prophylactic for feeding intolerance in preterm infants. A study on changes in intestinal normal flora

after erythromycin use is also needed.

Conflict of Interest

None declared.

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Original Article

The effect of cyanotic and acyanotic congenital heart disease on children's growth velocity

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Abstract

Background Congenital heart disease (CHD) can lead to failure to thrive. Decreased energy intake, malabsorption, increased energy requirements, and decreased growth factors (growth hormone/insulin-like growth factor 1 axis) are related to malnutrition and growth retardation in children with CHD.

Objective To compare the impact of cyanotic and acyanotic CHD on children's growth velocity (using the 2009 WHO growth velocity chart).

Methods This study was conducted in patients less than 24 months of age with CHD in the Pediatric Cardiology Specialist Unit Dr. Moewardi Hospital, Surakarta, Central Java, from December 2016 to February 2017. Subjects' weights were evaluated at the beginning of the study and two months later. Data were compared to the WHO Growth Velocity chart and analyzed by Chi-square test.

Results Of 46 patients with CHD (23 cyanotic, 23 acyanotic), 10 patients (21.7%) were identified with failure to thrive, i.e., < 5th percentile. Significantly more children with acyanotic CHD were in the >5th percentile for growth velocity than were children with cyanotic CHD (OR 5.600; 95%CI 1.038 to 30.204; P=0.032). Acute upper respiratory tract infection was not significantly associated with growth velocity (OR 2.273; 95%CI 0.545 to 9.479; P=0.253).

Conclusion Children with cyanotic CHD have 5.6 times higher risk of failure to thrive than children with acyanotic CHD. **[Paediatr Indones. 2017;57:159-62 doi: http://dx.doi.org/10.14238/pi57.3.2017.159-62]**.

Keywords: *congenital heart disease; growth velocity; failure to thrive*

Congenital heart disease (CHD) is the most prevalent structural malformation, constituting 25% of all congenital anomalies, and is on the forefront of global medical issues. CHD occurs in 0.5-0.8% of all births.[1-3] Children with CHD often face impaired growth and development. Chen *et al.* reported delays in growth and development among children suffering from CHD compared to their normal counterparts.[4] The so-called failure to thrive (FTT) is not a disease unto itself, but rather a symptom of a general pathway caused by one or more medical, psychosocial, or environmental issues leading to stunted growth in a child. Evaluation of children with slowed or stunted growth is a challenge to pediatricians.[5] The etiology of failure to thrive among CHD patients is still obscure. Many factors may contribute to such a condition, including caloric intake, malabsorption, increased use of energy, relative hypoxia, and endocrine adaptation.[5,6] We aimed to assess for a possible correlation between growth velocity and cyanotic and acyanotic CHD in children, using the 2009 WHO Growth Velocity Chart.

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Methods

This prospective cohort study was done to compare the impact of cyanotic and acyanotic CHD on children's growth velocity. This study was carried out in the Pediatric Cardiology Specialist Unit at Dr. Moewardi Hospital, Surakarta, Central Java, from December 2016 to February 2017. The target population was cyanotic and acyanotic CHD patients <24 months of age. Parents or guardians of patients who fulfilled the research criteria provided informed consent and participated by filling in the research forms. Patients with Down syndrome, immune deficiency, dysmorphia, severe sepsis, thyroid anomalies, and gastrointestinal congenital malformation, were excluded. All patients aged <24 months with cyanotic or acyanotic CHD who fulfilled the inclusion criteria were included consecutively. Subjects' weights were evaluated at the beginning of the study and taken prospectively two months later. The data were recorded in the 2009 WHO Growth Velocity Chart and failure to thrive (FTT) was defined to be < 5th percentile.

Data were processed and analyzed using *SPSS 20.0* software. Basic characteristics of the subjects (age, sex, type of congenital heart disease, and weight) were presented in numbers and percentages. Correlations between the independent and dependent variables were analyzed by Chi-square test. Confounding variables were analyzed by logistical regression multivariate statistical analysis.

Results

This study was performed on 46 patients diagnosed with cyanotic or acyanotic congenital heart disease. Of 56 initial patients, 10 were excluded due to Down syndrome and/or congenital hypothyroidism. **Table 1** shows that 58.7% of subjects were female and 41.3% were male. Most patients (65.2%) had no accompanying diseases (no acute upper respiratory tract infection, URI), whereas the remaining 34.8% had accompanying disease (acute URI). Most diagnoses of cyanotic CHD were tetralogy of Fallot (TOF) (30.4%), whereas most acyanotic CHD cases were diagnosed as atrial septal defect (ASD) (17.4%). In addition, 78.3% of patients did not have FTT, whereas the remaining 21.7% experienced FTT.

Table 1. Basic characteristics of subjects

Characteristics	N=46
Gender, n(%)	
Female	27 (58/7)
Male	19 (41.3)
CHD, n(%)	
Acyanotic	23 (50)
Cyanotic	23 (50)
Growth velocity, n(%)	
≤ 5th percentile	36 (78/3)
> 5th percentile	10 (21/7)
Accompanying disease, n(%)	
No URI	30 (65/2)
URI	16 (34/8)
Diagnoses	
Cyanotic CHD, n(%)	
TOF	14 (61)
TGA	3 (13)
Single atrium, single ventricle	1 (4)
Tricuspid atresia	1 (4)
DORV	1 (4)
Type-A truncus arteriosus	2 (9)
TAPVR	1 (4)
Acyanotic CHD, n(%)	
VSD	7 (30)
PDA	6 (26)
ASD	8 (35)
AVSD	1 (4)
PS	1(4)

ToF=tetralogy of Fallot, TGA=transposition of the great arteries, DORV=double outlet right ventricle, TAPVC=total anomalous pulmonary venous connection, VSD=ventricular septal defect, PDA=persistent ductus arteriosus, ASD=atrial septal defect, AVSD=atrial-ventricular septal defect,

In subjects with cyanotic CHD, 34.8% had <5th percentile growth velocity, while in the acyanotic group, only 8.7% had similarly poor growth. Chi-square analysis revealed a significant correlation between the cyanotic CHD and impaired growth velocity. Also, patients with cyanotic CHD had 5.6 times higher risk of FTT (OR 5.600; 95%CI 1.038 to 30.204; P=0.032) compared to acyanotic CHD patients (**Table 2**).

As shown in **Table 3**, 31.3% of URI patients had <5th percentile growth velocity compared to 16.7% of those without URI. Chi-square test revealed no significant association between URI and FTT (OR 2.273; 95%CI 0.545 to 9.479; P=0.253).

Table 2. Cyanotic and acyanotic CHD and growth velocity

Types of congenital heart disease	Growth velocity		Total (N=46)	OR (95%CI)	P value
	> 5th percentile (n=10)	≤5th percentile (n=36)			
Cyanotic (n=23)	8	15	23	5.600 (1.038 to 30.204)	0.032
Acyanotic (n=23)	2	21	23		

Table 3. URI and growth velocity

Accompanying disease	Growth velocity		Total (N=46)	OR (95%CI)	P value
	> 5th percentile (n=10)	≤5th percentile (n=36)			
URI (n=16)	5	11	16	2.273 90.545 TO 9.479)	0.253
No URI (n=30)	5	25	30		

Discussion

Children's growth velocity can be determined by the 2009 WHO Growth Velocity Chart.[7] The chart can also be used to assess children's vulnerability to FTT. A child is diagnosed with FTT if his/her weight is < 5th percentile. We compared FTT in children with cyanotic to acyanotic CHD. Failure to thrive in CHD patients has no clear etiology, but may be due to multiple contributing mechanisms, including inadequate caloric intake, decreased appetite, malnutrition caused by hypoxia, malabsorption due to venous congestion, increased use of energy, relative hypoxia, increased need for oxygen, endocrine adaptation, and recurring respiratory infection. Hypoxia is a result of an imbalance between the demand and supply of oxygen. In addition, CHD causes chronic hypoxia, resulting in low levels of IGF-1 in the patient's endocrine system.[6-11]

This analytical study was done in patients who underwent routine medical check-ups at the Pediatrics Cardiology Specialist Unit at Dr. Moewardi Hospital, Surakarta. In our study, there were more female CHD patients (58.7%) than males (41.3%). In contrast, Mahapatra et al. reported that more male patients (54.5%) suffered from CHD compared to females, with a ratio of 1.2 to 1. However, Batte *et al.* found more females with CHD (57.2%) than males.[12,13]

The most common cause of acyanotic CHD is VSD (35-30%), and that of cyanotic CHD is TOF (5-7%).[12] In our study, the most of our cyanotic CHD subjects had TOF (61%), and most of our acyanotic subjects had ASD (35%). Nasiruzzaman et *al.* reported that the most prevalent cyanotic CHD etiology was TOF (26%). Similar to our findings, Atwa *et al.* reported that the frequency of ASD in acyanotic CHD was higher (28.8%) than VSD (28.2%). However, Mahapatra et al. in India reported VSD as the most prevalent acyanotic CHD (36.3%), and TOF as the most prevalent cyanotic CHD (11.25%).[12,14,15]

In our study, 80% children with cyanotic CHD suffered from FTT. A previous study reported that 55.9% patients with CHD suffered from FTT.[16] Another study also reported FTT in 55% of neonates with VSD and TOF CHD.6 In addition, Harshangi *et al.* reported FTT complications among 56% of CHD patients.[17] Furthermore, Batrawy *et al.* reported that 60% cyanotic CHD patients suffered from growth anomalies.[9] Artiko *et al.* reported that patients with acyanotic CHD patent ductus arteriosus suffered from growth anomalies before a catheterization action was taken.[18] However, Nasiruzzaman *et al.* reported FTT occurrence in only 13% of children with CHD.[19]

We found that cyanotic CHD patients have a 5.6 times higher risk of FTT (OR 5.600; 95%CI 1.038 to 30.204) compared to acyanotic CHD patients. Batte et al. in Uganda found that CHD patients who suffered from growth anomalies, as determined by the 2006 WHO Nutritional Chart, also showed similar findings of increased FTT.[13]

In the ≥ 5th percentile category, 69.4% of patients did not suffer from acute URI and did not experience FTT, whereas 50.0% of patients in the < 5th percentile category suffered from URI

and experienced FTT. We noted that URI as an accompanying disease increased the tendency to have FTT (OR 2.273; 95%CI 0.545 to 9.479) but there was no statistically significant correlation between URI and growth velocity (P=0.253). Gabriela *et al.* reported that CHD patients who suffered from acute lower respiratory tract infection (ALRTI) cited bronchopneumonia as the most prevalent disease (86.6%).19 In addition, Medrano et al. reported that 13.5% of CHD patients were hospitalized due to respiratory tract infection.[20]

A limitation of our study was that other factors may cause failure to thrive, such as low body weight at birth, short pregnancy period, socioeconomic issues and malnutrition, none of which were evaluated in this study. In conclusion, cyanotic CHD poses higher risk of failure to thrive compared to acyanotic CHD.

Conflict of Interest

None declared.

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Original Article

The pediatric index of mortality 3 score to predict mortality in a pediatric intensive care unit in Palembang, South Sumatera, Indonesia

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Abstract

Background For critically ill patients in the pediatric intensive care unit (PICU), a scoring system is helpful for assessing the severity of morbidity and predicting the risk of mortality. The *Pediatric Index of Mortality* (PIM) 3 score consists of ten easy simple variables, so that the probability of death can be assessed prior to undergoing advanced therapies. The PIM 3 score in inexpensive and comprised of routine laboratory variables performed in PICU patients. In Indonesia, studies to validate the PIM 3 score have been limited.

Objective To evaluate the PIM 3 score for predicting the probability of death in the PICU, Dr. Mohammad Hoesin Hospital (MHH), Palembang.

Methods A prospective, cohort study was performed in the PICU, MHH, Palembang, from February to April 2016. The PIM 3 score was calculated within 2 hours of patients admission to the PICU by an android calculator application. PIM3 score and mortality were analyzed by Mann-Whitney test; calibration was performed by Hosmer-Lameshow goodness of fit test, discrimination was done by receiver operating characteristic (ROC) curve analysis; and standardized mortality ratio (SMR) was calculated.

Results During the study period there were 81 PICU patients, 69 children were included, ranging in age from 1,5 to 187 months. The overall mortality rate was 40,58%. The most common illnesses in our subjects were malignancy (17,4%), post non-thoracic surgery (14,5%), dengue shock syndrome (14,5%), respiratory disease (13%), and neurological disease (11,6%). Subjects' PIM3 scores ranged from 1,02% to 58,84%, with means of 26,08% in non-survivors and 13,05% in survivors. The SMR was 2,24, indicating that death was underpredicted. The AUC of 0,771 (95% CI of 0,651 to 0,891) indicated that the PIM3 score had good discrimination.

Conclusion In Mohammad Hoesin Hospital, Palembang, South Sumatera, the PIM 3 can be used to predict mortality in PICU patients, but the score should be multiplied by a factor of 2.24. This recalibration is needed due to the presumed lower standard of care at this hospital compared to that of the originating PIM 3 institutions in developed countries. **[Paediatr Indones. 2017;57:164-70; doi: http://dx.doi.org/10.14238/pi57.3.2017.164-70]**.

Keywords: *PIM3 score; probability of mortality; PICU MHH Palembang*

The hospital pediatric intensive care unit (PICU) has special staff and equipment to care for critically-ill or injured children aged 0 to 18 years (except neonates) in order to identify primary disease process and to support patients at risk for organ dysfunction.[1] PICU patients often have unclear prognoses and mortality rates may be dependent on PICU staff and procedures. A scoring system for illness severity is useful for objectively predicting the outcomes and prognoses of PICU patients.[2-8]

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Since 1980, there are various scoring systems have been used, such as the *Pediatric Risk of Mortality* (PRISM), P*ediatric Logistic Organ Dysfunction* (PELOD), and *Pediatric Index of Mortality* (PIM) tests. The newest version are PRISM III, PELOD 2, and PIM 3, respectively.[9-13] The first version of PIM was developed at 1997 in Australia and the United Kingdom. The second model, PIM 2, was developed using data collected from 13 PICU in 1997 and 1999 in Australia, New Zealand, and the United K ingdom.[14-15] The newest iteration, PIM 3, was developed in 2010-2011 by Straney *et al.* Discrimination of PIM3 was 0.88, compared to as high as 0.90 in PIM 2. The PIM 3 mortality risk was 3,9% in Scotland and England, and 2.9% in Australia and New Zealand. The difference may have been due to individual PICU performance or facilities, as well as the health status of the population.[13]

The PIM 3 scoring system has some advantages compared to other scoring systems. The PIM 3 consists of 10 simple variables which are easy to assess, can predict mortality before patients receive advanced therapy, are from routin PICU examinations, and are not prohibitively expensive to be used in a developing country.[14] In Indonesia, the PIM 3 model has yet to be validated, so we do not know if mortality predictions by PIM 3 in Indonesia are similar to those in developed countries. The objective of this study was to evaluate the utility of PIM 3 as a mortality predictor in the PICU, Mohammad Hoesin Hospital (MHH), Palembang, South Sumatera.

Methods

We conducted a prospective, cohort study in our PICU from February to April 2016. Inclusion criteria were critically ill PICU patients with reliable laboratory findings corresponding to the PIM 3 score variables, and whose parents provided informed consent. Patients who stayed in the PICU for less than 1 hours were excluded.

Collected data at admission included age, sex, weight, length, nutritional status, diagnosis, and PIM 3 variables such as systolic blood pressure and pupillary reaction to bright light. Our variables assessed were partial oxygen tension (PaO2) and FiO2 at the same time of PaO2 if oxygen was given by endotracheal tube, non-invasive ventilation, or headbox; base excess in arterial blood gas analysis, type of mechanical ventilation at any time during the first hour of PICU admission, elective admission to PICU, recovery from surgery or a procedure was the main reason for ICU admission, presence of low-risk diagnosis, high-risk diagnosis, or very high-risk diagnosis. Definitions of these variables and the scoring method were according to PIM 3 developers' guidelines.[13]

Scores were calculated using PIM 3 calculator application from *Australian and New Zealand Intensive Care Society* (AZNICS).[16] Data was entered into Microsoft Excel 2007 and analyzed using *SPSS version 16.0* software. We analyzed for an association between PIM 3 score and mortality. The performance of PIM3 score was assessed by calibration and discrimination. Calibration evaluated PIM 3 at different risks of mortality, and was assessed by Hosmer–Lemeshow table. Standardised mortality ratio (SMR) was calculated to mean probability of death and the ratio of observed to expected death rates. Discrimination evaluated how well PIM distinguished between patients who survived and died, and was assessed using the area under the curve from the ROC plot. The study was approved by the Ethics Committee of the Sriwijaya University Medical School.

Results

Over the study period, 81 patients were admitted to the PICU, but data were collected from only 69 subjects who qualified based on inclusion criteria. Of these 69 subjects, 28 (40.58%) died. Subjects' median age was 89 months, with the highest mortality in the 60-119 month group (45.83%). The majority of subjects were boys (59.4%). About a half (43.5%) of the children had good nutritional status, while non-survivors had malnutrition and undernutrition (50%). The most common underlying disease for PICU admission was malignancy (17.4%), but the mayor cause were central nervous system and burns (8/8 and 1/1, respectively. Demographic features and clinical course of subjects related to outcome are provided in **Table 1**.

Subjects' PIM 3 scores ranged from 1.02% to 58.84%, with mean and median scores of 18.34% and 13.05%, respectively. Most subjects were in the 5-20% score interval. No subjects had scores of less than

Table 1. Characteristics of patients related to outcome

Characteristics	All patients (N=69)	Non-survivors (n=28)	Survivors (n=41)
Gender, n(%)			
Male	41 (59.4)	15	26 (63.4)
Female	28 (40.6)	13	15 (53.6)
Age group, n(%)			
< 12 months	10 (14.5)	4	6 (14.6)
12-59 months	24 (34.8)	9	15 (36.6)
60-119 months	24 (34.8)	11	13 (31.7)
> 120 months	11 (15.9)	4	7 (17)
Nutritional status, n(%)			
Malnutrition	4 (5.8)	2	2 (4.9)
Undernutrition	22 (31.9)	11	11 (26.8)
Good nutrition	30 (43.5)	8	22 (53.6)
Overweight	4 (5.8)	3	1 (2.4)
Obesity	9 (13.0)	4	5 (12.2)
Diagnosis, n(%)			
Malignancy	12 (17.4)	7	5 (12.2)
Post-surgical procedure besides thoracic surgery	10 (14.5)	1	9 (21.9)
Dengue shock syndrome	10 (14.5)	5	5 (12.2)
Respiratory system	9 (13.0)	2	7 (17)
Central nervous system	8 (11.6)	8	0
Cardiogenic shock/CHF/arrythmia	6 (8.7)	3	3 (7.3)
Endocrine-metabolic	4 (5.8)	1	3 (7.30
Post-thoracic surgery	4 (5.8)	0	4 (9.7)
Sepsis/septic shock	2 (2.9)	0	2 (4.9)
Snake bite	2 (2.9)	0	2 (4.9)
Emergency hypertension	1 (1.4)	0	1 (2.4)
Burns	1 (1.4)	1	0

Table 2. Distribution of PIM 3 scores related to outcomes

PIM 3 score interval by group, n(%)	All patients (N=69)	Outcomes	
		Non-survivors (n=28	Survivors (n=41)
1-5%	12 (17.4)	3	9 (22)
5-20%	36 (52.2)	11	25 (61)
20-30%	8 (11.6)	4	4 (9.7)
>30%	13 (18.8)	10	3 (7.3)

Table 3. PIM3 score related to outcome

Outcome	N	Median PIM 3 score (range), %
Non-survivors	28	21.4 (2.7-58.8)
Survivors	41	11.4 (1.0-53.9)

1%. Higher PIM 3 score indicated higher probability of mortality. The PIM 3 score intervals and subject outcome are shown in **Table 2**.

Mean and median PIM 3 scores in non-survivors were 26.1% and median 21.4%, respectively. For survivors, mean and median of PIM 3 scores were 13.1% and 11.4%, respectively (**Table 3**). Mann-Whitney test revealed that median PIM 3 score in non survivors were significantly higher compared to the value in survivors (P=0.0001).

Table 4 shows the SMR calibration of the PIM 3 model based on four PIM 3 score intervals of 1-5%, 5-20%, 20-30%, and >30%. The overall SMR was 2.24. The expected mortality rate based on PIM 3 score was 18.1%, less than the actual percentage observed (40.5%).

The PIM 3 score was divided in quartiles of risk, by range interval of 14.45. The SMRs in each quartiles of risk group were >1, which meant mortality was underpredicted. The AUC curve analysis showed a good discriminatory ability of the PIM 3 score in 44.38-58.84% interval group to distinguish between survivors and non-survivors (AUC>70%) (**Table 5**).

Table 6 shows that SMRs in overall demographic and clinical course group were >1, except for predicting mortality in the subgroups of post-surgical procedure other than thoracic surgery, others illnesses (snake bite, diabeticum ketoasidosis, and post thoracic surgery), and respiratory system.

Table 4. Observed and expected of mortality based on PIM 3 score intervals by group

PIM 3 interval by group	Mean PIM 3 (%)	N	Non-survivors		Survivors		Chi-square value of Hosmer Lemeshow Goodness of fit	SMR
			Observed, n	Expected, n(%)	Observed, n	Expected, n(%)		
1-5%	3.3	12	3	0.4 (3.3)	9 (75)	11.6 (96.7)	16.9	7.5
5-20%	12.11	36	11	4.4 (11.43)	25 (69.4)	31.6 (87.7)	9.9	2.5
20-30%	23-34	8	4	1.8 (22.5)	4 (50.0)	6.2 (77.5)	2.69	2.22
>30%	45.14	13	10	5.87 (45.15)	3 (23.08)	7.13 (54.8)	2.9	1.70
Total		69	28 (40.6)	12.47 (18.06)	41 (59.4)	56.53 (81.9)	19.34	2.24

SMR=standardized mortality ratio

Table 5. Calibration of PIM 3 score based on quartiles of risk

Quartiles of risk (%)	Mean PIM 3 score	Total, n(%) (N=69)	Non-survivors (n=28)		Survivors (n=41)		SMR	AUC (95% CI)
			Observed, n	Expected, n(%)	Observed, n(%)	Expected, n(%)		
1-15.45	9.21	44 (63.77)	11	4.05 (0.09)	33 (75)	39.95 (90.8)	2.7	0.715 (0.585 to 0.845)
15.46-29.91	22.76	12 (17.4)	7	2.73 (22.7)	5 (41.67)	9.27(77.25)	2.56	0.613 (0.435 to 0.791)
29.92-44.37	37.78	8 (10.14)	5	2.27 (37.8)	2 (33.3)	3.73 (62.2)	1.76	0.648 (0.417 to 0.878)
44.38-58.84	51.44	6 (8.69)	5	3.08 (51.3)	1 (16.67)	2.92 (48.67)	1.62	0.751 (0.578 to 0.924)

Table 6. Performance of PIM3 score related to age, nutritional status, and primary diagnosis

Variables	Mean PIM 3 score	n	Non-survivors (n=28)		Survivors (n=41)		Chi-square Hosmer Lemeshow Goodness of fit	SMR
			Observed, n(%)	Expected, n(%)	Observed, n(%)	Expected, n(%)		
Age group, n(%)								
< 12 months	27.53	10	4	2.75 (27.5)	6 (60)	7.25 (72.5)	0.57	1.25
12-59 months	16.85	24	9	4.04 (16.8)	15 (62.5)	19.9 (83.2)	6.08	2.23
60-119 months	19.45	24	11	4.7 (19.45)	13 (54.2)	19.3 (80.4)	8.44	2.34
> 120 months	10.78	11	4	1.2 (10.78)	7 (63.3)	9.8 (89)	6.53	3.33
Nutritional status, n(%)								
Malnutrition	27.42	4	2	1.09 (27.4)	2 (50)	2.91 (72.7)	0.76	1.83
Undernutrition	18.92	22	11	4.16 (18.9)	11 (50)	17.8 (81)	11.25	2.64
Good nutrition	16.86	30	8	5.06 (16.86)	22 (73.3)	24.9 (83.1)	1.7	1.58
Overweight	17.74	4	3	0.7 (17.8)	1 (25)	3.3 (82.5)	7.56	4.3
Obesity	18.04	9	4	1.62 (18)	5 (55.56)	7.38 (82)	3.5	2.47
Diagnosis, n(%)								
Malignancy	18.75	12	7	2.25 (18.75)	5 (41.7)	9.75 (81.2)	10.03	3.11
Post-surgical procedure besides thoracic surgery	15.88	10	1	1.59 (15.9)	9 (90)	8.41 (84.1)	0.21	0.63
Dengue shock syndrome	22.57	13	6	2.93 (22.6)	7 (53.8)	10 (76.9)	3.22	2.04
Respiratory system	24.25	9	2	2.18 (24.2)	7 (77.8)	6.82 (75.8)	0.01	0.92
Central nervous system	17.77	8	8	1.42 (17.7)	0	6.58 (82.2)	30.49	5.64
Cardiology	15.77	6	3	0.95 (15.8)	3 (50)	5.05 (84.2)	4.43	3.16
Others*	11.50	11	1	1.26 (11.5)	10 (80)	9.74 (88.5)	0.05	0.79

*Others: snake bite, diabetic ketoacidosis, post-thoracic surgery

Table 7 and **Figure 1** show the area under the curve of PIM 3 score ROC analysis. The AUC of the PIM 3 score was 0.771 (95% CI 0.67 to 0.75), and the AUC of the PIM 3 quartiles of risk was >0.7 (0.715). An AUC of 70-80% is considered to be accurate for predicting death and survival.

Discussion

Our study investigated 69 PICU patients at MHH, Palembang, South Sumatera, during a-3 months period, in order to evaluate the performance of PIM 3, in terms of calibration and discrimination ability

Table 7. Discrimination of PIM 3 and PIM 3 quartiles of risk as related to death

Variables	AUC	SE	95%CI
PIM 3 score	0.771	0.061	0.651 to 0.891
PIM 3 quartiles of risk	0.715	0.003	0.585 to 0.845

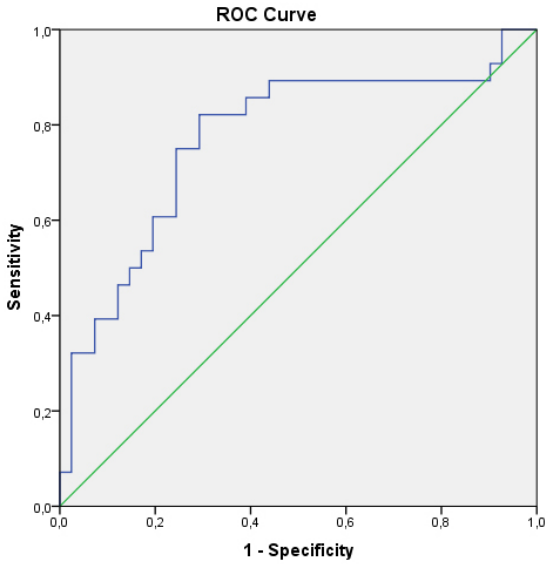

Figure 1. ROC Curve Analysis for PIM3 Scores

compare to those observed in developed countries. The prevalence of mortality in our PICU was 40.58%, similar to that of Honna *et al* (45.7%).[17] The prevalence of PICU mortality was also similar to that from India in 2011 (46.2%),[18] but higher than in Pakistan in 2006 (28.7%),[19] Iran in 2008 (15%),[20] and Egypt in 2013 (8.5%).[21]

The PIM 3 model uses score variables to get a percentage of mortality probability. Overall, in the originating studies, the PIM 3 mortality risk were 3.9% in Scotland and United Kingdom and 2.9% in Australia and New Zealand. Validation of the PIM3 model has never been done in a developing country, but validation of the second iteration of PIM (PIM 2) was done in in Iran, Pakistan, India, and Africa.[14,19-21]

We found that PIM 3 scores in non-survivors ranged from 2.73% to 58.84% with a mean of 26.08%. For survivors, mean PIM 3 score was 11.38%, ranging from 1.02-53.9%. Most subjects (52.17%) were in the 5-20% range. Higher PIM 3 score means higher mortality probability. Of those with PIM 3 scores >30% score, 76.9 died, while 23.1% survived. Mann-Whitney test revealed a statistically significant difference in PIM 3 score between non-survivors and survivors (P=0.0001). This mortality probability was much higher than documented rates at others PICUs where validation of ordinary prognostic scores had been undertaken. As such, the standard of care in our PICU may be worse then PICUs in developed countries. The following factors may influence PICU performance: differences of clinical characteristics, demographic population, health status, human sources (nurse to patient ratio, human exhaustion factor, subjective factors in evaluating PIM 3 score, the doctor's ability in treat and make accurate and timely decisions related to clinical condition, as well as nursing skill in getting arterial blood specimens), and validity of the laboratory for measuring based excess.[22] Mohammad Hoesin Hospital, Palembang, as a teaching hospital, placed senior of pediatric residents as doctors on duty in the emergency room and PICU under pediatric intensivist supervision. The skill of these doctors could subjectively influenced the measures of PIM 3's variables, such as the size of pupillary reaction to bright light, the decision to gave mechanical ventilation before the first hour in PICU, and skill in setting mechanical ventilation support and FiO2 for patients. These factors could influence the final PIM 3 score, even though we have trained aur staff in clear operational procedures to standardize evaluations in an effort to minimize bias. Nevertheless, some subjects were stabilized in a tertiary hospital or private clinic prior to referral to MHH, which may also have influenced the PIM 3 score variables.

The calibration of the PIM3 model using SMR was calculated by dividing the number of observed deaths by the number of expected deaths. Chi-square statistical analysis was performed with the formula: $\Sigma(O-E)2/E$, in which is O=observed and E=expected, for survivors and non-survivors in each interval group. Then we used Hosmer and Lemeshow test for goodness-of-fit based on the four PIM3 score interval group of 1-5%, 5-20%, 20-30%, and >30%. The SMRs for all interval was >1, ranging from 1.7-2.2, except for the 1-5% group which had SMR 7.5, indicating that the actual mortality was 7.5 times higher than expected in the 1-5% interval group. Multiple factors may have influenced these results, such as poor referral system, delayed initial therapy,

or complications which could have changed outcome, such as hospital-acquired infection, malnutrition caused by hospitalization, or ventilator-associated pneumonia. The ability of PIM 3 to predict mortality was 18.07%, which was less than the actual observed mortality of 40.5%. The overall SMR was 2.24, which meant that the PIM 3 model underpredicted deaths in our facility. As such, the mortality probability was 2.24 times higher in MHH compared to the original PIM 3 score. Other studies in developing countries like India, Pakistan, and Egypt reported SMRs from PIM 2 scores of 3.3, 1.57, and 1.92, respectively.[19,21] On the other hand, a Japanese study reported PIM 2 SMR <1 (0.77), which meant the score had overpredicted mortality.[23]

The discrimination was evaluated by AUC. Discrimination is considered to be very good for ROC >0.9, good for 0.80–0.90, and fair for 0.70–0.80. The AUC was calculated as 0.771 (95%CI 0.651 to 0.951), lower than AUC in the original places where PIM 3 score was undertaken. However, AUCs were found in developed countries as follows: Australia 0.91, New Zealand 0.90-0.93, United Kingdom 0.85, and Scotland 0.84-0.86.14 Studies of PIM2 in developing countries in Africa showed good discrimination with AUC values of 0.841 (95%CI 0.78 to 0.90),[24] Pakistan 0.81 (95%CI 0.75 to 0.87), and Iran 0.795 (95%CI 0.715 to 0.875).[20]

In conclusion, PIM 3 score has quite good calibration in our set-up. The PIM 3 score can be used in PICU, MHH, Palembang by correcting for the expected probability of death by multiplying the original PIM3 score by 2.24. This calibration needs to be done due to the presumed lower standard of care at MHH compared to the standards in the originating PIM 3 institutions. The standard of care may be influenced by multiple factors, such as clinical characteristics, demographic population, health status, human resources, medical equipment, good laboratory, and more.

Conflict of Interest

None declared.

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Original Article

Effectiveness of ferric sodium edentate supplementation in children with lead poisoning

Praevilia Salendu, Nurhayati Masloman, Sarah M. Warouw

Abstract

Background Lead is a harmful toxin that affects human health worldwide, especially in children. Lead poisoning remains a global problem both in developed and developing countries. The *Centers for Disease Control and Prevention* (CDC) recommends nutritional intervention with iron supplementation to efficiently control high lead levels. Iron supplementation in the form of sodium ferric ethylene-diaminetera-acetic acid/ ferric sodium edentate (NaFeEDTA) is highly bioavailable.

Objective To determine the effectiveness of ferric sodium edentate (NaFeEDTA) on lead levels in children woaj ;ead [pospmomg.

Methods This interventional, analytical study, had a one group pretest-posttest design, and was done on children in four elementary schools in the Talawaan District, North Minahasa Regency, Manado, North Sulawesi, from August to November 2014. Inclusion criteria were elementary students aged 6-9 years with lead poisoning (lead levels ≥ 10 μg/dL) and good nutritional status. Subjects were given NaFeEDTA 115.4 mg (15 mg elemental iron) at a dosage of 3 mg/kgBW/day elemental iron given between meal times. Iron supplementation was given daily per oral route for 12 weeks. Descriptive analysis was used to analyze the characteristics of the study sample. Pre- and post-test analyses were done with paired T-tests. Significance level was P <0.05.

Results In this study, 39 children met the inclusion criteria and consisted of 19 boys and 20 girls. Their mean age was 8.43 (SD 0.44) years. Pre-test and post-test blood lead levels was 36.18 μg/dL and 5.22 μg/dL, respectively.There was a significant reduction in mean blood lead levels after administration of NaFeEDTA (P <0.0001).

Conclusion In children with lead poisoning, blood lead levels are significantly reduced after 12 weeks of NaFeEDTA supplementation. **[Paediatr Indones. 2017;57:171-5; doi: http://dx.doi.org/10.14238/pi57.4.2017.171-5]**.

Keywords: *lead poisoning; blood lead levels; NaFeEDTA*

Lead is a harmful toxin that affects human health. In children, lead can cause decreased level of intelligence (IQ points), decreased learning ability, impaired growth and hearing, anemia, as well as conduct disorder/attention and behavior problems. Currently, lead contamination occurs everywhere. Lead poisoning in Indonesia is thought to come from various sources, such as leaded gasoline, paint, vegetables, fertilizers, and other sources. Blood lead level is the gold standard for determining its effect in the blood. The *Centers for Disease Control and Prevention* (CDC), the *American Academy of Pediatrics* (AAP), and several national and international organizations have established that blood lead levels of >10 μg/dL are considered to be lead poisoning.[1,2,3] In some large cities in Indonesia,

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35.4 to 90% of children have blood lead levels >10 μg/dL. Iron is an important metal substitution target of Pb2+. At the molecular level, iron deficient children have increased expression of the divalent metal transporter 1 (DMT-1) in the duodenum, in order to increase iron absorption. However, lead absorption can also increase in these children.[4,5] One month of iron supplementation in children with iron deficiency anemia was proven to decrease blood lead levels. The CDC recommends nutritional intervention with iron supplementation to control high blood lead levels. Iron supplementation in form of ethylenediamineteraacetic acid ferric sodium / ferric sodium edentate (NaFeEDTA) has a high bioavailability. Content of EDTA in NaFeEDTA increased the bonding of minerals from blood, one of them is lead.[6,7] The aim of this study was to evaluate the effectiveness of ferric sodium edentate (NaFeEDTA) supplementation on decreasing blood lead levels in children with lead poisoning.

Methods

This analytic, interventional study had a one-group, pretest-posttest design. The study was conducted in four elementary schools located in the Talawaan District of North Minahasa Regency, Manado, North Sulawesi, from August to November 2014. Subjects were aged 6-9 years, with blood lead levels ≥ 10 μg/dL, the established cut-off for diagnosing lead poisoning.1 Inclusion criteria were students with lead poisoning (≥ 10 μg/dL), good nutritional status based on the 2000 CDC growth charts, and normal hemoglobin (Hb) level (Hb ≥ 11 g/dL). Exclusion criteria were students with fractures, infections such as runny nose, cough, or diarrhea, high fever within the three consecutive days before blood specimens were taken, concomitant diseases such as renal or liver disease, infections that needed a long period of treatment such as tuberculosis, tumors, history of blood abnormalities or acute bleeding in the three months prior to the study, or those who took iron supplementation in the three months prior to the study. We also excluded students who had received iron supplementation, were hypersensitive to iron preparations, experienced bleeding within the past 3 months including from worm infestations, fractures, infections, or concomitant diseases such as kidney, liver, tuberculosis, and malignancy.

Subjects' parents provided informed consent and filled questionnaires about the state of their child's health. Drop-out criteria were recurrent vomiting, inability to swallow the supplement according to the adjusted dosage, hypersensitivity reactions, moving out of town, quit from the study, or refused to continue the therapy. Subjects were collected by purposive sampling and underwent blood lead level measurements, pre- and post-treatment). Subjects were then given NaFeEDTA 115.4 mg (15 mg elemental iron) at a dosage of 3 mg/kgBW/day elemental iron given between meal times. Iron supplementation was given daily per oral route. If a subject vomited within less than 1 hour of taking the supplement, the iron supplement could be given at the same dose as before. But if the vomiting persisted or a subject had diarrhea as an adverse effect, that subject was considered to have dropped out of the study. The iron supplementation also had to be stopped if adverse effects such as severe gastrointestinal problems, nausea, vomiting, and diarrhea persisted. After 12 weeks of NaFeEDTA supplementation, subjects' complete blood counts and blood lead levels were evaluated.

Subjects provided 10 mL blood specimens taken from the median cubital vein in a sterile fashion. Five mL of the blood was kept in an EDTA-anticoagulant tube for complete blood counts, at the Laboratorium Klinik Manado, and the other 5 mL was stored in a heparin tube and sent to Jakarta for blood lead level testing.

Descriptive analysis of the subjects' characteristics was reported in distributive tables. Parametric data was used to calculate mean, standard deviation (SD), and 95% confidence interval (CI). Pretest and posttest analyses were done with paired T-test. Results were considered to be statistically significant for P values <0.05. Data were processed with *SPSS for Windows version 22* software. The study was approved by the Ethics Committee of the Sam Ratulangi University Medical School, Manado.

Results

From August to November 2014, 46 children from four elementary schools were identified. After purposive sampling by physical examination and laboratory findings, 39 children met the inclusion criteria. The mean age of subjects was 8.43 (SD 0.44) years. More girls than boys had lead poisoning. Subjects' mean body weight was 24.56 (SD 3.49) kg. Antropometric measurements showed that all subjects had good nutritional status (**Table 1**). Subjects' complete blood counts before supplementation showed normal levels of hemoglobin, leukocytes, and platelets according to age (**Table 1**).

Table 1. Subjects' characteristics

Characteristics	All patients (N=69)
Mean age (SD), years	8.43 (0.44)
Gender, n	
Female	20
Male	19
Mean body weight (SD), kg	24.56 (3.49)
Mean body height (SD), cm	125.37 (6.46)
Mean Waterlow score (SD), %	99.15 (6.81)
Mean hemoglobin (SD), g/dL	12.96 (0.53)
Mean leucocytes (SD), cells/mm^3	10,305 (2,332.37
Mean platelets (SD), cells/mm^3	337,948 (43,668.57)

Paired T-test revealed a significant difference in mean blood lead levels, before and after iron supplementation [36.18 (SD 11.97) *vs.* 5.22 (SD 2.03) μg/dL, respectively; (P<0.0001)] (**Table 2**).

had been done in the Talawaan District.[8] Our study was conducted in a subset of children who had blood lead levels > 10 μg/dL in the previous study.

All subjects had good nutritional status, as shown by normal anthropometric parameters, in order to eliminate confounding micronutrient deficiencies that could affect the blood lead level. The US CDC showed that nutritional status is negatively related to blood lead levels in children. Good nutritional status can help prevent toxicity caused by lead.[9]

Subjects' hemoglobin levels were evaluated to check for anemia. Anemia is known to be an effect of lead poisoning but it may also be caused by iron deficiency, chronic disease, parasitic infection, and other conditions related to blood abnormalities. Anemia was not found in any subjects.[10] Lead poisoning and iron deficiency anemia in children had been studied before, especially in high risk populations, such as in children living in lead mining areas. However, lead poisoning was found to have a greater effect on neurotoxicity than on heme synthesis.[11,12]

Subjects' leukocytes were evaluated to eliminate a confounding factor of infectious disease. Leukocyte levels in this study were in the normal range. Lead poisoning may increase leukocyte levels with leukocytosis occurring especially in the monocyte and neutrophil series.[13]

A previous study in the Talawaan District reported a high mean blood lead level of 25.8 μg/dL in children.8 We found that the mean blood lead level had increased to 36.18 μg/dL. Chronic

Table 2. Serum lead levels (initial and after NaFeEDTA administration

Serum lead levels	Mean (SD), µg/dL	95%CI	P value
Initial	36.16 (11.97)	32.30 to 40.06	0.0001
After NaFeEDTA administration	5.22 (2.03)		

Discussion

The Talawaan District is located in the North Minahasa Regency, and consists of 11 villages. The 4 elementary schools chosen for the study were located near one and others, and isolated, thus the lead exposure were from the environment, not from vehicles. A previous study on blood lead levels in children aged 6-8 years

exposure, resulting in an accumulation of lead in the bloodstream, and the tendency of increasing blood lead level as a function of age, where older children are more exposed to lead from the environment, activities, and food, may have lead to this increase only 2 years later. Chronic exposure to lead can cause high blood lead levels after a long period of time.[14,15] Children are particularly prone to the effects of lead exposure.

The risk of lead exposure in villages is different from that in cities. People who live near gold mining areas are susceptible to lead poisoning over a long time frame.[16] A gold mine is located near the Talawaan District (approximately 2 km away). Trucks transporting stones and supplies to process the gold pass through the villages, spreading lead-containing dust. Motorcycles, as main vehicle in the area, are also major contributors to the pollution problem. The villages in our study also had a similar demographic, as they are all located near a river that passes through the mining area.

Studies from 12 gold mines in Brazil showed toxicity or lead poisoning in children living nearby. The destruction of lead-rich stones and the amalgamation process in gold production, released toxic minerals including lead, which had not been water soluble prior to mining. Chronic exposure of lead-containing dust was also a cause of lead poisoning. Gold mine production resulted in unnecessary mineral residues, called tailing. The disposal of this tailing is an environmental problem, because it contains metal components, such as Pb, Hg, Zn, As, and Cd that pollutes land and water.[16] The CDC and WHO reported on deaths caused by lead poisoning, where 97% of children living near a gold mine in Nigeria had blood lead levels of ≥45 μg/dL.[17] However, we cannot conclude that subjects' elevated blood lead levels in the Talawaan District are related to the presence of nearby gold mines.

In our study, 26 children had mild lead poisoning (10-40 μg/dL), 11 had moderate (40-60 μg/dL), and 2 children had severe lead poisoning (63 μg/dL and 74 μg/dL, respectively). In home visits and investigations into the potential source of pollution, no sources were found other than in their living environment.

The influence of lead poisoning in children may be minimized by improving the intake of iron and calcium. Lead competes for the same metalloprotein-binding sites as iron and calcium, thus adequate levels of these minerals may decrease the effects of lead in the body. Children with mild lead poisoning are advised to improve their nutritional intake and begin iron supplementation.[18]

Medical therapies should be given to children manifesting symptoms of lead poisoning or children with blood lead levels ≥45 μg/dL.[19] In our study, 7 had blood lead levels ≥45 μg/dL, hence, medical and chelating therapies, as well as iron supplementation should be given as soon as possible to decrease the blood lead levels, as recommended by the CDC.[19] Children with severe lead poisoning should undergo complete blood screening and blood lead level evaluation weekly and then monthly. Children with manifestations of lead poisoning should be admitted to the hospital and given chelating therapy. All children with blood lead level > 20 μg/dL should be moved away from the source of lead, as recommended by the CDC.[18] However, the situation must be discussed with the community and government.

In our study, 3 months of administration of iron in the form of NaFeEDTA, orally at a dose of 3 mg/BW/day was adequate to significantly decrease serum lead in children with lead poisoning ($P<0.0001$). Furthermore, a healthy, iron-rich diet is required. NaFeEDTA was not given in conjunction with lead chelation, as chelation inhibits iron absorption.[20] In vitro trials in rats showed a decrease in serum lead levels in the blood-brain barrier after six weeks of NaFeEDTA administration.[21] In children with lead poisoning and iron deficiency, the administration of NaFeEDTA can decrease serum lead levels. A Bangalore study showed a decrease of serum lead levels from ≥10 μg/dL after iron fortification for six days per week for six months.[22]

This study is the first to assess the effectiveness of NaFeEDTA administration in children with lead poisoning. Our results provide a basis for communities and health care providers to influence decision-making of the local government, as elevated serum lead levels in Talawaan children needs immediate attention. A limitation of this study was that we could not determine the precise source of lead pollution in the area, despite the suggestion of such sources by the subjects' high lead levels which exceeded the safe threshold set by CDC. Also, our subjects did not undergo complete radiography examinations, according to CDC recommendations for evaluating children with lead poisoning, nor did they have periodic evaluations of serum lead levels, prior to initiating treatment. Medical therapy is needed in children with lead poisoning accompanied by symptoms. Recording and calculation of diet and nutrition, including all food containing iron, was also not done in this study. However, only children with good nutritional status were included in our study. In

conclusion, blood lead levels are significantly reduced after 12 weeks of NaFeEDTA supplementation in children with lead poisoning.

Conflict of Interest

None declared.

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Original Article

Focused group discussion with health care staff improves breastfeeding rates in hospitalized infants

Agnes Yunie Purwita Sari, Rosalina Dewi Roeslani, Rinawati Rohsiswatmo

Abstract

Background Improving breastfeeding in sick infants is essential. During the neonatal care, health care staff play an important role in promoting breastfeeding. Therefore, it is important to study in depth how healthcare staff can improve breastfeeding practice in sick neonates.

Objective To compare breastfeeding rates in sick infants before and after a focused group discussion (FGD) of health care staff on how to improve breastfeeding.

Methods This study was an operational study using FGD and in-depth interviews as an intervention. A fish bone diagram was used to assess problems that may prevent mothers from breastfeeding their sick infants. Breastfeeding achievement was compared before and after the FGD.

Results Of 257 sick infants, 177 subjects were in the before FGD group and 80 subjects were in the after FGD group. Significantly more after FGD subjects were breastfed during hospitalization than before FGD subjects [97.5% *vs.* 82.9%, respectively; (X^2 =9.43; P=0.002)]. Breastfeeding initiation within 0-4 hours of birth was also significantly higher in the after FGD group [10 (12.5%) *vs.* 6 (3.5%), respectively; (X^2 = 52.5; P<0.001)]. The solutions for breastfeeding problems were: 1) support of hospital management, 2) support of healthcare workers for breastfeeding mothers, 3) support of husbands and families for breastfeeding mothers, 4) financial support, 5) other factors such as level of care and consistent FGD events, and 6) a prospective cohort study.

Conclusion The FGD with health care staff significantly increases breastfeeding achievement during infant hospitalization, and accelerated breastfeeding initiation. A fish bone diagram is used to effectively assess the problems with breastfeeding programs for sick babies. **[Paediatr Indones. 2017;57:187-93 ; doi: http://dx.doi.org/10.14238/pi57.4.2017.187-93]**.

Keywords: *breastfeeding; sick babies; qualitative study; FGD*

Although breast milk has been shown to reduce morbidity and mortality in infants, optimal achievement of breastfeeding in sick neonates has not been realized. Evidence has shown that nutrients in breast milk reduce the incidence of necrotizing enterocolitis, chronic lung disease, retinopathy of prematurity, and infection, as well as shortening the length of hospital stay, especially for preterm infants.[1-7] Despite the benefits of breast milk, breastfeeding rates in sick neonates are far from meeting expectations. According to 2013 data from the Neonatology Division, Department of Pediatrics, Cipto Mangunkusumo Hospital, the proportion of newborns who received breast milk during hospitalization was only 5%, and only 2% of those received exclusive breastfeeding. About 14.7% of newborns did not receive breast milk within the first 4 weeks of life, despite the breastfeeding achievement target of 90% for well infants and 20% for sick infants. Therefore, efforts to improve breastfeeding achievement in sick infants are essential.

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Measures to improve breastfeeding achievement have been done but their effectiveness was insufficient, hence, a new approach is required. One measure to improve breastfeeding achievement was health care staff training using a structured, clinical, objective-referenced, problem-oriented, integrated, organized (SCORPIO) method.[8] This training was conducted for a year with a focus on breastfeeding and included all health care staff in Dr. Cipto Mangunkusumo Hospital, Jakarta. However, the neonatal division reported fluctuative and below target rates of breastfeeding in sick infants. As a new strategy to complement healthcare staff training, a focused group discussion (FGD) of the staff was undertaken to improve breastfeeding achievement in sick newborns.

The aim of this study was to determine the effectiveness of FGD with health care staff for increasing breastfeeding rates in sick infants. Breastfeeding achievement in infants was compared before and after the FGD. In addition, factors affecting breastfeeding achievement and difficulties faced by the staff in improving breastfeeding achievement were also determined.

Methods

This study was operational research, using qualitative and quantitative methods. After receiving approval from the Medical Ethics Committee, we collected retrospective data from medical records and the neonatal registry of the Neonatal Unit, Dr. Cipto Mangunkusumo Hospital, from January to March 2015 to determine the rate of breastfeeding achievement. Then, the FGD, combined with in-depth interviews of health care staff, were conducted. During the FGD, a qualitative research method and fish bone diagram were used to gain information and to assess problems related to breastfeeding implementation in sick infants.[9] After FGD with healthcare staff, quantitative data were collected to determine the rates of breastfeeding in sick infants.

The target population of this study was sick, hospitalized neonates, admitted to the level 2 or level 3 Neonatal Care Unit from January to March 2015 (before FGD) and December 2015 (after FGD), which was within one month after the FGD. The subjects were allocated through non-probability sampling. The minimum number of required subjects was 75 participants for each group.[10] All subjects from a previous pilot study were enrolled as the before FGD group (177 newborns). Neonates who had not been given enteral nutrition or were discharged without a doctor's approval were excluded.

The 9 health care staff included breastfeeding coordinators, nurses in charge of breast milk delivery, dieticians, and a pediatric nutrition supervisor, as the FGD and in-depth interview participants. In addition to healthcare staff, parents were included in the FGD and in-depth interviews. The allocation for FGD respondents was through purposive sampling.

The *Statistical Package for Social Sciences (SPSS)* for Windows software was used for data analysis, comprising univariate, bivariate, and multivariate analyses. The data are summarized in diagrams, tables, and text.

Results

In general, the demographic and clinical characteristics of subjects were not significantly different between groups. However, significantly more after FGD subjects required ventilation support and inotropic drugs than did the before FGD subjects. The characteristics of subjects are shown in **Table 1**.

After the FGD, breastfeeding achievement in sick babies during hospitalization significantly increased. Of the total sample, 78 (97.5%) subjects after FGD received breast milk compared to 145 (82.9%) of those before FGD ($X^2=9.43$; $P=0.002$) (**Table 2**).

A significantly higher percentage of the after FGD group received breast milk during their first week of hospitalization than did the before FGD group [74 (92.5%) *vs.* 131 (74.9%), respectively; ($X^2=9.75$; $P=0.002$)] (**Table 3**).

Before FGD, 46.9% of infants received partial breastfeeding, and 25.1% babies did not receive breast milk at all. However, after FGD, the majority of subjects received partial breastfeeding (72.5%) and predominant breastfeeding (17.5%), significantly more than in the before FGD group ($X^2=36.76$; $P<0.001$) (**Table 4**). However, much fewer from the after FGD group (0.5%) received exclusive breastfeeding compared to the before FGD (22.3%).

Table 1. Demographic and clinical characteristics of subjects (N=257)

Characteristics	Before FGD (n=177)	After FGD (n-80)	X^2 or F	P value
Maternal education, n(%)				
Well-educated	39 (22)	13 (16.3)	X^2=0.81	0.32
Under-educated	138 (78)	67 (83.8)		
Commuting time to hospital, n(%)			X^2=1.97	0.37
<1 hour	60 (33.9)	34 (42.5)		
1-2 hours	69 (39)	29 (36)		
>2 hours	48 (27.1)	17 (21.3)		
Mean maternal age (SD), years	28.9 (6.27)	27.9 (6.12)	F=1.25	0.21[a]
Median parity (IQR) [N=256, missing 1 (0.6%)]	1 (0-2)	1 (0.5-1.5)		0.43[b]
Gender, n(%)			X^2=0.20	0.65
Male	80 (45.2)	33 (41.3)		
Female	97 (54.8)	47 (58.8)		
Level of neonatal care, n(%)			X^2=2.61	0.14
Level 2	110 (62.1)	58 (72.5)		
Level 3	67 (37.9)	22 (27.5)		
Mean gestational age (SD), weeks	35.0 (3.48)	34.9 (3.84)		
Gestational age by group, n(%)			X^2= 0.54	0.76
<32 weeks	28 (15.8)	15 (18.8)	F=1.21	0.23[a]
32-36 weeks	76 (42.9)	31 (38.8)		
≥37 weeks	73 (41.2)	34 (42.5)		
Mean birth weight (SD), grams	2,264 (764,06)	2,213 (873)		
Birth weight by group, n(%)			X^2=3.88	0.27
<1,500 grams	31 (17.5)	21 (26.3)	F=0.120	0.89[a]
1,500-1.999 grams	36 (20.3)	13 (16.3)		
2.000-2.499 grams	41 (23.2)	13 (16.3)		
≥1,500 grams	69 (39)	33 (41.3)		
Median APGAR score at 1st minute (IQR) [N=243, missing 14 (5.45%)]	7 (5.5-8.5)	7 (5.5-8.5)		
Median APGAR score at 5th minute (IQR) [N=243, missing 14 (5.45%)]	9 (8-10)	9 (8.5-9.5)		0.17[b]
Types of delivery, n(%)			X^2=0.001	0.97
Vaginal	55 (31.1)	25 (31.3)		
C-section	122 (68.9)	55 (68.7)		
Twins, n(%)	14 (7.9)	4 (5)	X^2=0.35	0.56
Persistant ductus arteriosus, n(%)	16 (9)	4 (5)	X^2=0.75	0.39
Hypotension requiring inotropic drugs, n(%)	4 (2.3)	8 (10)	X^2==5.72	0.02
Resusitation at birth, n(%) [N=253, missing 4 (1.6%)]	99 (57.2)	47 (58.8)	X^2==0.008	0.82
Ventilation support, n(%)			X^2=10.60	0.005
None	75 (42.4)	18 (22.5)		
Non-invasive ventilation	75 (42.2)	50 (62.5)		
Invasive ventilation	27 (15.3(	12 (15)		

[a]=T-test; [b]=Mann-Whitney test; IQR=interquartile range

Table 2. Breastfeeding achievement for sick newborns during hospitalization

Breastfeeding during hospitalization	Before FGD (n=175)	After FGD (n=80)	X^2	P value
Received breast milk, n(%)	145 (82.9)	78 (97.5)	9.43	0.002
Did not receive breast milk, n(%)	30 (17.1)	2 (2.5)		

N valid=255, missing 2 (0.8%)

In addition to the quantity of breast milk, breastfeeding initiation time after birth was significantly lower in the after FGD group than in the before FGD group, as shown in **Table 5**. There were 10 (12.5%) after FGD subjects who received breastfeeding within the first 4 hours of admission compared to 6 (3.5%) before FGD subjects (X^2=52.5; P<0.001). However, significantly more before FGD subjects than after FGD subjects were in the >4-24 hour initiation time category.

In this study, factors that were significantly different between the breastfed and non-breastfed groups were as follows: level of hospitalization, birth weight, gestation, APGAR score at the first and fifth minutes, resuscitation at birth, ventilation support, and FGD. These findings are described in **Table 6**.

Further logistic regression analysis revealed that only level of neonatal care and FGD affected breastfeeding achievement in sick infants. Infants hospitalized in level 2 neonatal care had 5.18 times (95%CI 2.19 to 12.28; P<0.001) higher odds to achieve breastfeeding compared to those hospitalized in level 3. Infants hospitalized after FGD had a 6.31 times (95%CI 1.43 to 27.87; P=0.01) higher odds to achieve breastfeeding compared to those hospitalized before the FGD was conducted. Level of care and FGD explained 20% of the variation in breastfeeding achievement in sick infants during hospitalization (Nagelkerke R2=0.20).

The qualitative section of the study was an applied, naturalistic study, in order to understand the health care staff's experiences, expectations, and attitudes towards a program for promoting breastfeeding for sick infants. Purposively recruited, the respondents were 9 staff responsible for feeding and providing education to promote breastfeeding. In addition, parents were also invited to participate

Table 3. Breastfeeding achievement during the first week of hospitalization

Breastfeeding during the first week of hospitalization	Before FGD (n=175)	After FGD (n=80)	X^2	P value
Received breast milk, n(%)	131 (74.9)	74 (92.5)	9.75	0.002
Did not receive breast milk, n(%)	44 (25.1)	6 (7.5)		

N valid=255, missing 2 (0.8%)

Table 4. The quantity of breast milk given during the first week of hospitalization

Breastfeeding during the first week of hospitalization	Before FGD (n=175)	After FGD (n=80)	X^2	P value
Exclussive breastfeeding	39 (22.3)	2 (0.5)	36.76	<0.001
Predominant breastfeeding	10 (5.7)	14 (17.5)		
Partial breastfeeding	82 (46.9)	58 (72.5)		
Did not receive breast milk, n(%)	44 (25.1)	6 (7.5)		

N valid=255, missing 2 (0.8%)

Table 5. Breastfeeding initiation time for sick infants before an

Breastfeeding initiation time	Before FGD (n=173)	After FGD (n=80)	X^2	P value
0-4 hours	6 (3.5)	10 (12.5)	52.5	0.001
>4-24 hours	61 (35.3)	15 (18.8)		
>1-4 days	48 (47.7)	35 (43.8)		
5-7 days	16 (9.2)	13 (16.3)		
>1-2 weeks	12 (6.9)	4 (5)		
3 weeks or more	30 (17.3)	3 (3.8)		

N valid=253, missing 4 (1.6%)

in the study.

Focused group discussion using a fish bone diagram was an effective method to improve breastfeeding achievement in sick infants. During the FGD, 10 topics were discussed. Through the use of a fish bone diagram, six factors were identified as the causes of poor breastfeeding achievement in sick infants. Short-term and long-term applicable solutions were proposed by the staff through six tools in the fish bone diagram shown in **Figure 1**.

Table 6. Factors affecting breastfeeding for sick infants during hospitalization, as grouped by breastfeeding status

Hospitalized subjects (N=255)	Breastfed (n=223)	Not breastfed (n=32)	P value/t/X^2
Education, n(%)			
[N=255, missing 2 (0.8%)]			
HIgh	45 (88.2)	6 (11.8)	X^2=0.06; P=0.85
Low	178 (87.3)	26 (12.7)	
Commuting time, n(%)			X^2=1.68; P=0.43
[N=255, missing 2 (0.8%)]			
<1 hour	83 (89.2)	10 (10.8)	
1-2 hours	87 (88.8)	11 (11.2)	
>2 hours	53 (82.8)	11 (17.2)	
Mean maternal age (SD), years	28.38 (6.12)	29.53 (6.53)	t=0.99; P=0.33
Median parity (IQR)	1 (0.5-1.5)	1 (0-2)	0.21[a]
[N=256, missing 1 (0.4%)]			
Gender, n(%)			X^2=0.02; P=0.87
[N=255, missing 2 (0.8%)]			
Male	97 (86.6)	15 (13.4)	
Female	126 (88.1)	17 (11.9)	
Level of neonatal care, n(%)			X^2=20.75;
[N=255, missing 2 (0.8%)]			P<0.001
Level 2	158 (94.6)	9 (5.4)	
Level 3	65 (73.9)	23 (26.1)	
Mean gestational age (SD), weeks	35.39 (3.49)	33.06 (3.79)	t=-3.49; P=0.001
Mean birth weight (SD), grams		1,843 (842)	t=-3.12; P=0.002
[N=255, missing 2 (0.8%)]	2,307 (779)		
Types of delivery, n(%)			X^2=0.001;
[N=255, missing 2 (0.8%)]			P=0.97
Vaginal	69 (87.3)	10 (12.7)	
C-section	154 (87.5)	22 (12.5)	
Median APGAR score at 1st minute (IQR)	7 (5.5-8.5)	6 (4.1-7.8)	P=0.04[a]
[N=243, missing 14 (5.45%)]			
Median APGAR score at 5th minute (IQR)	9 (8-10)	8 (6.5-9.5)	P=0.005[a]
[N=243, missing 14 (5.45%)]			
Multiple pregnancy, n(%)	14 (77.8)	4 (22.2)	X^2=0.84; P=0.36
[N=255, missing 2 (0.8%)]			
Persistant ductus arteriosus, n(%)	16 (4.2)	3 (15.8)	X^2=0.007
[N=255, missing 2 (0.8%)]			
Hypotension requiring inotropic drugs, n (%)	11 (91.7)	1 (8.3)	X^2=0.21; P=0.65
[N=254, missing 3 (1.2%)]			
Resuscitation at birth, n (%)	120 (82.2)	26 (17.8)	X^2=6.98; P=0.008
[N=251, missing 6 (2.4%)]			
Ventilation support, n(%)			X^2=19.68;
[N=255, missing 2 (0.8%)]			P<0.001
None	86 (94.5)	5 (5.5)	
Non-invasive ventilation	111 (88.8)	14 (11.2)	
Invasive ventilation	26 (66.7)	13 (33.3)	
Health staff FGD, n(%)			X^2=9.43;
Before	145 (82.9)	30 (17.2)	P=0.002
After	78 (97.5)	2 (2.5)	

[a]=Mann-Whitney test

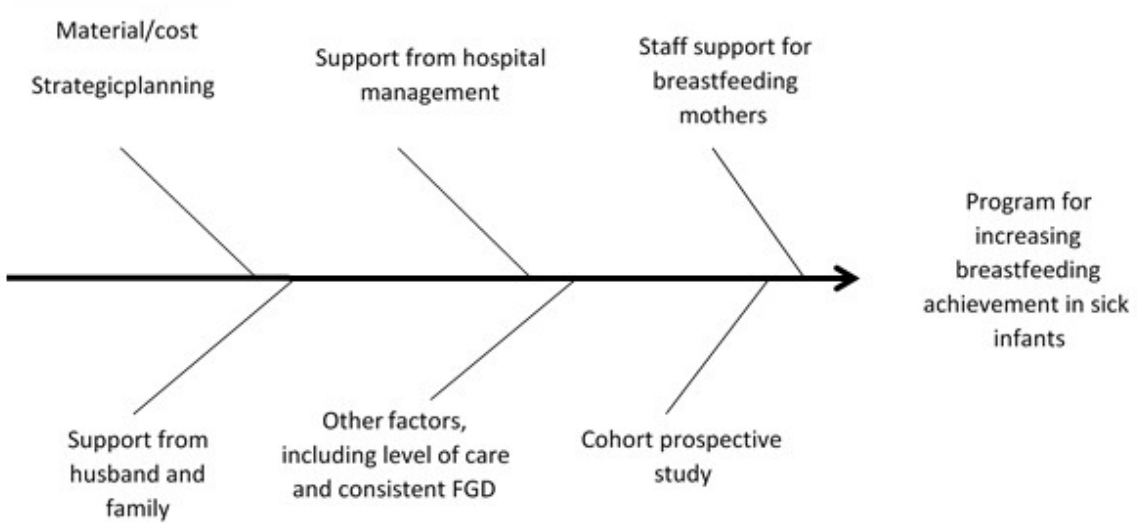

Figure 1. Fish bone diagram from the FGD to improve breastfeeding achievement in sick infants

Discussion

Subjects' characteristics were comparable between the before and after FGD groups, except for hypotension requiring inotropic drugs and ventilation support. However, multivariate analysis revealed that hypotension and ventilation support were not significantly associated with breastfeeding achievement during hospitalization.

In addition to FGD, breastfeeding achievement in sick infants was influenced by level of care in the neonatal unit. However, both FGD and level of care only explained 20% of the variation in breastfeeding achievement in sick infants. Therefore, 80% of the variation in breastfeeding achievement between groups must be due to other factors that require further investigation.

This study was an operational research project, using both quantitative and qualitative approaches. The qualitative component, including FGD and in-depth interviews, revealed more information about problems faced by healthcare staff in promoting breastfeeding for sick babies. A fish bone diagram facilitated the problem-mapping, analysis, and problem-solving processes in this study. The role of the quantitative method was to determine the effect of FGD intervention on breastfeeding.

Further continuous improvements are essential. Because of the cross-sectional study design, in which independent and dependent variables were measured at the same time, the temporal relationship between those variables was not easily determined. As such, a prospective cohort study with continuous data surveillance about breastfeeding achievement and its influencing factors is required. In addition, in this study the amount of breast milk given was measured semi-quantitatively. Recording the amount of breast milk given to sick babies during hospitalization is necessary. Moreover, FGD in this study did not involve physicians, who play an important role in deciding the types of feeding, including breastfeeding, for sick infants. In the future, FGD involving doctors and other health staff is required.

In conclusion, FGD with healthcare staff increases breastfeeding achievement in sick infants during their hospitalization. In addition, FGD accelerates breastfeeding initiation, as well as promoted partial and predominant breastfeeding. Education and training combined with FGD of healthcare staff is a effective strategic model to promote breastfeeding for sick babies. As a qualitative study, FGD reveales problems related to breastfeeding for hospitalized infants. A fish bone diagram effectively describes the problems and promoted problem-solving processes to improve breastfeeding achievement in sick neonates.

Conflict of interest

None declared.

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Original Article

Waist circumference and insulin levels in obese children

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Abstract

Background Childhood obesity is one of the most serious public health challenges of the 21st century. Its prevalence has increased at an alarming rate. Overweight and obese children are prone to obesity in adulthood and to developing non-communicable diseases (NCDs) like diabetes and cardiovascular diseases at a younger age. Increased waist circumference has been shown to contribute to the risk of metabolic syndrome in obese adults.

Objective To assess for a correlation between waist circumference and insulin level in obese children.

Methods In this cross-sectional study, obese children aged 6-10 years were included by consecutive sampling. We excluded children with infectious disease, malignancy, dyslipidemia, type 2 diabetes mellitus, or those who had not fasted before the blood draw. Subjects underwent waist circumference and fasting blood glucose measurements. Serum insulin levels were examined by enzyme-labeled chemiluminescent immunometric assay, after subjects had fasted for 10-14 hours. Data were analyzed by correlation analysis.

Results Subjects had a mean waist circumference of 80.2 (SD 7.2) cm and mean insulin level of 10.70 (SD 7.5) μIU/mL . Pearson's correlation test revealed a significant, moderately positive correlation between waist circumference and elevated insulin level (r=0.45; P=0.006).

Conclusion Waist circumference and insulin level have a significant, moderate, positive correlation in obese children. As such, waist circumference may be a simple method for early detection of hyperinsulinemia, as a risk factor for metabolic syndrome. **[Paediatr Indones. 2017;57:194-7 ; doi: http://dx.doi.org/10.14238/pi57.4.2017.194-7]**.

Keywords: *waist circumference; insulin level; obese; children*

Childhood obesity is one of the most serious public health challenges of the 21st century. The problem is global and is steadily affecting many low- and middle-income countries, particularly in urban settings. The prevalence has increased at an alarming rate. Globally, in 2013 the number of overweight children under the age of five, was estimated to be over 42 million.[1] The 2013 Indonesian National Health Survey (Riskesdas) found that the prevalence of obesity in children aged 5-15 years was 8.8%,[2] while the prevalence of obesity in the pediatric outpatient clinic at Sanglah Hospital was 21.7%.[3]

Childhood obesity is associated with a higher chance of premature death and disability in adulthood. Overweight and obese children are more likely to remain obese into adulthood and to develop non-communicable diseases (NCDs) like diabetes and cardiovascular diseases at a younger age.[4,5] Recent study has shown the importance of

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hyperinsulinemia, insulin resistance, and impaired glucose tolerance in promoting atherosclerosis. It is also well established in adults that obesity is related to hyperinsulinemia and insulin resistance although the precise mechanism underlying the relationships remains controversial.[4-6]

Indicators of body composition are strongly associated with metabolic changes, and it is important to verify their relationship with components of metabolic syndrome and with insulin resistance.[2,7,8] The body mass index (BMI) is the tool most commonly used to estimate overweight and obesity in children and adults. However, BMI has its limitations because increases may be related to increased fat-free mass. Also, the relationship between BMI and fat varies according to age, sex, and degree of sexual maturity. At the end of the 1990s, waist circumference (WC) was noted to be a simple screening tool, as abdominal fat may be a better predictor cardiovascular risk.[9,10]

Waist circumference and waist-to-hip ratio (WHR) are indices of body fat distribution, and have been associated with morbidity and mortality in adults. However, their prognostic value in children has been inconclusive and no data are available on tracking from childhood to adulthood.[6] Thus, we examined a possible correlation between waist circumference and insulin level in obese children.

Methods

This cross-sectional study was conducted from August to December 2015 in five elementary schools in Denpasar, Bali. The study was approved by the Ethics Review Board at Udayana University, Bali. Subjects were obese children aged 6–10 years and selected by consecutive sampling. Sample size was calculated based on a correlation study formula with type one error of 5% ($Z\alpha=1.96$), and type 2 error of 20% ($Z\beta=0.842$). The minimal correlation (r) was 0.42. The minimum required number of subjects was calculated to be 42 children.

Subjects included in this study were children aged 6-10 years and got the informed consent from their parents to be involved in this study. Children with infectious disease, malignancy, type 2 diabetes mellitus, or those who had not fasted before blood specimens were obtained, were excluded.

Anthropometric measurements included body weight, height, and waist circumference. Waist circumference was measured using a meter tape at the midpoint between the lowest rib and the end point of the iliac crest. Body mass index (BMI) was calculated based on body weight in kilograms divided by height in meters-squared (kg/m2). Obesity was determined using the WHO 2006 growth standard chart. Children were classified as obese for BMI Z scores $\geq +2$ SD. Fasting insulin levels were determined from subjects' serum specimens by Prodia Laboratory after subjects had fasted for 10-14 hours. An enzyme-labeled chemiluminescent immunometric assay (*Immulite*® 2000 Analyzer Systems, Siemens Healthcare Diagnostics Products Ltd., Llanberies, Gwynedd, United Kingdom) was used to measure serum insulin, with results expressed in a numeric scale of microunit units/mL, to the nearest 0.1 microunit/mL.

Data was processed and analyzed with *SPSS 16* software. Descriptive data were presented in text and tables. Pearson's correlation test was used for data a nalyses.

Results

During the study period, 1,946 elementary school students aged 6-10 years underwent anthropometric screening, of whom 170 were classified as obese. One hundred twenty-four children refused to participate in this study, and one child was excluded because his insulin level was too low. Characteristics of subjects are shown in **Table 1**. Mean age of children in this study was 8.8 years, mean of waist circumference was 80.2 cm, and mean of insulin level was 10.7μIU/mL. We found a significant, moderate, positive correlation between waist circumference and insulin level (**Table 2** and **Figure 1**).

Table 1. Characteristics of subjects

Characteristics	N=45
Males, n(%)	29 (64)
Mean age (SD), years	8.8 (1.3)
Mean body weight (SD), kg	45.9 (9.2)
Mean body height (SD), cm	136.6 (9.4)
Meang waist circumference (SD), cm	80.2 (7.2)
Mean body mass index (SD), kg/m^2	24.4 (2.5)
Mean insulin level (SD), μIU/mL	8.8 (1.3)

Table 2. Pearson's correlation test results

		Waist circumference
Insulin level	R	0.45
	P	0.006

Discussion

We observed that more male subjects (64%) were obese than female subjects. The prevalence of obesity in Medan elementary school children in 2007 was 60% in males and 39% in females.[11] Lone *et al.* also reported that of 262 obese children aged 4-10 years, 60% were male and 40% were female.[5] Dewi *et al.* reported that more male (83%) than female children in an urban area were prone to obesity.[12]

Obesity in childhood is associated with risk factors of cardiovascular disease later in life. In a population-based study, obesity during childhood was the strongest risk factor for metabolic syndrome (MetS). MetS is defined as a combination of conditions, including hyperlipidemia, insulin resistance, hyperglycemia, hypertension, and abdominal obesity, which exist in a constellation of interconnected physiological, biochemical, clinical, and metabolic factors.13 Abdominal fat is considered to be the key determinant of metabolic risk, since the pro-inflammatory adipokines secreted by visceral fat are related to increased blood pressure, dyslipidemia, and insulin resistance.[14] Various studies have shown that obese children have low HDL cholesterol, high triglycerides and insulin levels, but normal blood glucose levels, suggesting that glucose intolerance may develop later than other syndrome abnormalities.[15] Waist circumference is an indicator of central obesity, and is considered to be a risk factor for cardiovascular disease, stroke, and type 2 diabetes in adults.

Romero-Velarde *et al.* reported that mean insulin level and waist circumference in obese children were 16.7 μIU/mL and 86.1 cm, respectively. Waist circumference had a significant positive correlation with insulin level in obese children (r=0.58).10 Similarly, Bedogni *et al.* suggested that waist circumference was the best single predictor of insulin level in obese children (r=0.16), compared to BMI (r=0.09).6 In addition, Lone *et al.* found that mean insulin level and waist circumference in obese children were 12.7 μIU/mL and 70.1 cm, respectively.[5] Also, we found mean insulin level of 10.7 μIU/mL and mean waist circumference of 80.2 cm. Waist circumference had a positive correlation with insulin level in obese children in our study.

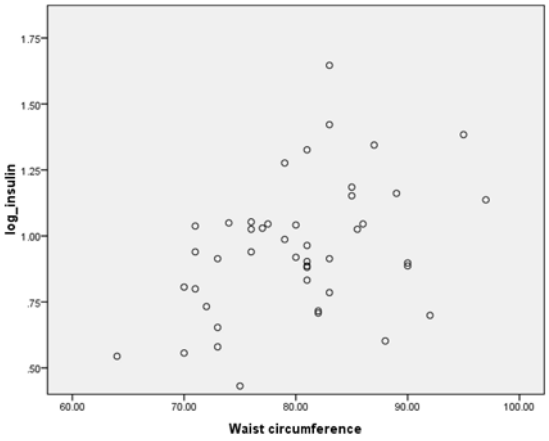

Figure 1. Scatterplot of waist circumference and insulin level

In conclusion, there is a moderately positive correlation between waist circumference and insulin level in obese children. As such, waist circumference can be used as an early detection method for the occurrence of hyperinsulinemia and insulin resistance as risk factors of metabolic syndrome.

Conflict of Interest

None declared.

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Original Article

Intravenous paracetamol and patent ductus arteriosus closure in preterm infants

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Abstract

Background Indomethacin and ibuprofen are the drugs of choice for closure of patent ductus arteriosus (PDA) in preterm infants. However, intravenous preparations are of limited availability in Indonesia. Circumstantial evidence has shown that intravenous paracetamol may be an alternative therapy for PDA closure in premature infants.

Objective To evaluate the effect of intravenous paracetamol on PDA closure in preterm infants.

Methods A before-and-after study was conducted between May and August 2014 in Cipto Mangunkusumo General Hospital, Jakarta in preterm infants with hemodynamically significant PDAs, as established by echocardiography using the following criteria: duct diameter >1.4 mm/kg, left atrium to aorta ratio >1.4, and mean velocity in the left pulmonary artery >0.42 m/s or mean diastolic velocity in the left pulmonary artery >0.2 m/s. Subjects, aged 2 and 7 days, received intravenous paracetamol (15 mg/kg every six hours) for 3 days. Paired T-test was used to compare pre-intervention PDA diameter to those assessed at 24 hours after the intervention and at 14 days of life.

Results Twenty-nine subjects had a mean gestational age of 30.8 weeks and mean birth weight of 1,347 grams. Nineteen (65.5%) patients had closed PDAs at the day 14 evaluation, 1 experienced PDA reopening, and 9 had failed PDA closure. No liver toxicity was identified. Mean duct diameters before, 24 hours after the intervention, and at 14 days of life were 3.0, 0.9, and 0.6 mm, respectively (P<0.0001).

Conclusion Intravenous paracetamol seems to be reasonably effective for PDA closure in preterm infants. **[Paediatr Indones. 2017;57:198-203 ; doi: http://dx.doi.org/10.14238/pi57.4.2017.198-203]**.

Keywords: *intravenous paracetamol; patent ductus arteriosus; preterm neonates*

Patent ductus arteriosus (PDA) is a common clinical problem encountered in preterm neonates. The three management approaches for PDA in preterm infants are conservative monitoring, medical treatment, or surgical ligation, depending on the hemodynamic significance of the shunt and associated comorbidities.[1] With regards to medical treatment, indomethacin and ibuprofen are anti-prostaglandin drugs widely used for PDA closure in preterm infants. However, these drugs are may have severe adverse events, such as acute kidney injury associated with indomethacin use, or gastrointestinal bleeding with ibuprofen.[2] Another issue is the limited availability of intravenous preparations, which are often required for sick infants who are contraindicated for oral or enteral intake. Therefore, in this setting, preterm infants with significant PDAs often have to proceed directly to surgical ligation, which carries higher risks of morbidity and mortality.[3]

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Paracetamol, a widely available non-steroidal anti-inflammatory drug, also has an anti-prostaglandin effect, but through a different mechanism from indomethacin, ibuprofen, or other non-selective cyclooxygenase (COX) inhibitors. Paracetamol acts as a peroxidase inhibitor in prostaglandin synthesis.4 In previous case series involving a limited number of subjects, both oral[5-12] and intravenous preparation[12-15] of paracetamol showed satisfactory results for PDA closure, with no liver toxicity. Moreover, two randomized, double-blind, control trials showed that oral paracetamol was better than oral ibuprofen.[16,17] However, as evidence for intravenous paracetamol was from anecdotal case reports, so far it has not been recommended as a drug of choice for PDA closure in neonates, although it may serve as an alternative drug, particularly for preterm infants with contraindications for oral or enteral intake.

This study aimed to evaluate the effect of intravenous paracetamol on PDA closure in preterm infants.

Methods

A before-and-after study was conducted between May and August 2014 in the neonatal intensive care unit (NICU), Cipto Mangunkusumo General Hospital, Jakarta. We enrolled infants aged 2 to 7 days, with gestational age less than 36 weeks and birth weight less than 2,000 grams, who had PDAs and were contraindicated for enteral intake. A PDA was defined as hemodynamically significant (hs-PDA) if the duct diameter was more than 1.4 mm/kg, left atrium to aorta (LA/Ao) ratio was more than 1.4, mean velocity in the left pulmonary artery was more than 0.42 m/s or mean diastolic velocity in the left pulmonary artery was more than 0.2 m/s. Based on a previous study, the sensitivity and specificity of these criteria were above 90%.20 The 2D and Doppler echocardiography were performed using a *Philips® HD11XE* ultrasound machine with a 12 MHz transducer. Duct diameter was measured by parasternal short axis view. We excluded infants with major congenital anomalies, other congenital heart diseases (except persistent foramen ovale), persistent pulmonary hypertension of the newborn (PPHN), hyperbilirubinemia requiring exchange transfusion, neonatal hepatitis before intervention, and infants who died before completing the intervention. This study was approved by the Research Ethics Committee, University of Indonesia Medical School, Jakarta. Written informed consent was obtained from parents before intervention.

All subjects underwent baseline clinical examinations such as assessment of gestational age based on the New Ballard Score (NBS), birth weight, biological sex, Apgar scores, vital signs, and routine blood tests. We administered bolus intravenous paracetamol (15 mg/kg) every 6 hours for 3 days. Echocardiographic evaluations to assess PDA diameter were performed 24 hours after the first intervention, 24 hours after the second intervention, and at 14 days of life. The second 3-day intervention with the same drug and dose was given if the child's PDA persisted after the first intervention. A complete closure was defined as a closed PDA at 14 days of life. Otherwise, the treatment was considered to have failed. We also recorded any adverse events, including mortality. We stopped the intervention if patients developed a significant elevation in serum transaminase levels. The study would be terminated if there was a subject died due to neonatal hepatitis.

Clinical characteristics and echocardiographic findings were described as a proportion or mean (standard deviation, SD), as appropriate. Paired T-test was used to compare PDA diameters before intervention to those 24 hours after the first or second intervention, and on day 14. A P value of less than 0.05 was considered to be statistically significant. All statistical analyses were performed using *SPSS for Windows version 18.0*.

Results

A total of 171 preterm infants were treated in our hospital, but only 127 (74.3%) infants underwent echocardiography examinations due to other clinical conditions of hemodynamic significance, such as respiratory disorders, history of asphyxia, tachycardia, heart enlargement based on chest X-ray, gastrointestinal bleeding, or other conditions suggesting contraindications of oral therapy. Echocardiography was also not conducted on babies in critical condition in the emergency room. In addition, three infants with congenital anomalies, two infants with complex congenital heart

diseases (transposition of the great arteries and complete atrioventricular septal defect), and two infants with PPHN were excluded.

Thirty-six infants (28.3%) were found to have patent ductus arteriosus/PDA. Twenty-nine (80.6%) subjects completed the entire study protocol (**Figure 1**). The clinical characteristics and echocardiographic findings are summarized in **Table 1**. After the first intervention, 21 (61.8%) subjects were defined to have PDA closure. After the second intervention, of 13 subjects with closure failure after the first intervention, 4 (11.8%) subjects had PDA closure. At 14 days of life, 19 (55.9%) subjects had been successfully treated, and 10 (34.5%) subjects had treatment failure, including one subject with reopening of the PDA.

The mean PDA diameters significantly decreased between before intervention and after the first intervention, after the second intervention, and at 14 days of life. However, the mean PDA diameter did not significantly decrease between after the second intervention and at 14 days of life (**Table 2**). Seven infants died because of severe sepsis and multiple organ system failure. Three cases experienced intraventricular hemorrhage (IVH) grade III/IV and one case had gastrointestinal bleeding. No necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), or retinopathy of prematurity (ROP) were observed. Subjects' median aspartate transaminase (AST) level was 16 (4-42) U/L and median alanine transaminase (ALT) level was 8 (4-20) U/L.

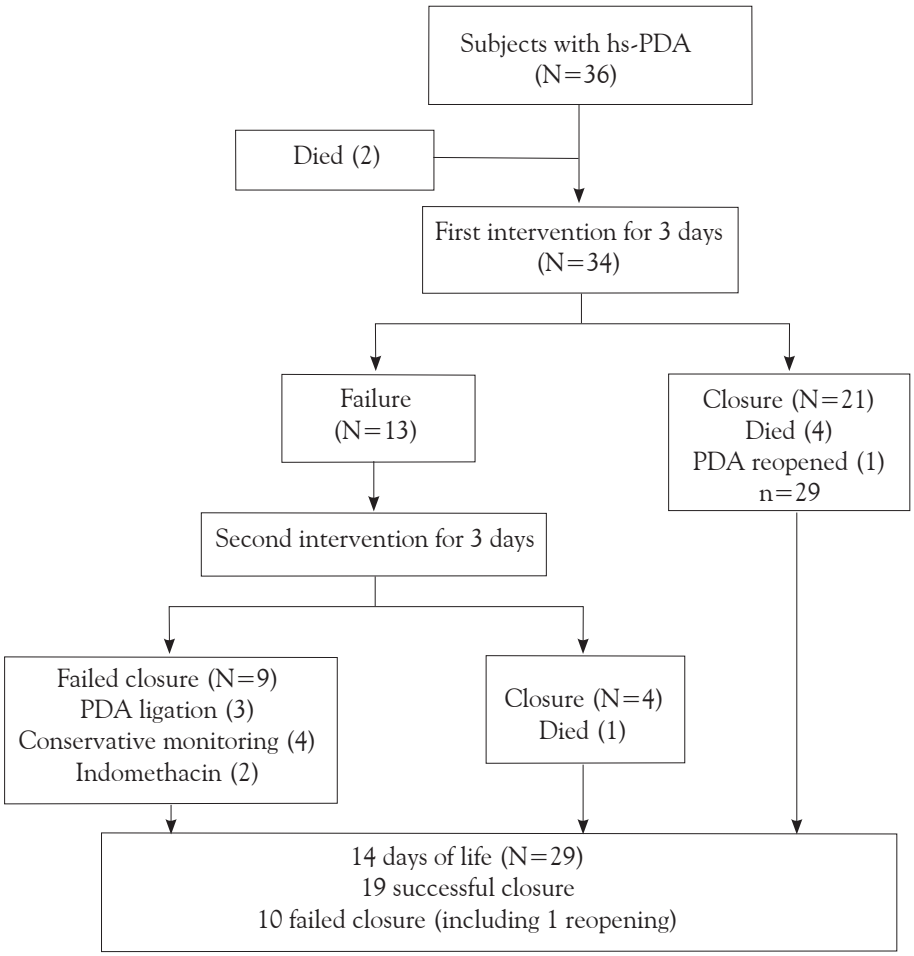

Figure 1. Flow diagram of study

Table 1. Clinical characteristics and echocardiographic findings

Characteristics	N=36
Gender	
Male, n	18
Female, n	18
Gestational age	
Mean (SD), weeks	30.8 (2.52)
< 32 weeks, n	25
33-36 weeks, n	11
Birth weight	
Mean (SD), grams	1347 (285.3)
< 1,500 grams, n	23
1,500-2,000 grams, n	13
Median (range) chronological ag at enrollment, hours	72 (49-165)
Median Apgar score (range)	
At 1 min	6 (1-9)
At 5 min	8 (3-10)
Platelet < 60,000/mL, n	2
Platelet 60,000-150,000/mL, n	10
Neonatal asphyxia, n	10
Respiratory distress syndrome grade III/IV, n	12
Apneua of premturity, n	19
Heart rate > 180 bpm, n	15
Murmur, n	9
Heart enlargement, n	6
Hyperbilirubinemia requiring phototherapy, n	12
Supportive therapy	
Nasal CPAP, n	20
Mechanical ventilation, n	16
Antibiotic	
Ampicillin-sulbactam + gentamicin, n	8
Pipercillin-tazobactam + amikacin, n	25
Metropenem, n	3
Total parenteral nutrition,	3
Blood transfusion, n	6
Echocardiographic findings	
Mean duct diameter (SD), mm	3.0 (0.54)
Mean LA/Ao ratio (SD)	1.8 (0.32)
Mean flow velocity in left pulmonary artery (SD), m/s	0.8 (0.25)
Mean diastolic flow velocity in left pulmonary artery (SD), m/s	0.4 (0.19)

Table 2. The differences of PDA diametera at before, after first, and after second intervention, and at 14 days of life

Characteristics	n	Mean diameter (SD), mm	Difference (SD)	P value
Before intervention	34	3.0 (0.55)	2.1 (1.04)	<0.0001
After first intervention		0.9 (1.18)		
Before intervention	34	3.0 (0.55)	2.4 (1.00)	<0.0001
After second intervention		0.6 (0.61)		
Those who failed after first intervention	13	2.2 (0.70)	0.8 (1.04)	0.016
After second intervention		1.4 (1.11)		
Before intervention	29	3.0 (0.57)	2.4 (0.91)	<0.0001
Chronological age 14 days		0.6 (0.98)		
After first intervention	17	0.9 (1.19)	0.3 (0.87)	0.059
Chronological age 14 days		0.6 (0.98)		
After second intervention	12	0.6 (1.03)	0.0 (0.52)	0.630
Chronological age 14 days		0.6 (0.98)		

Notes: 34=total number of infants enrolled in the first intervention, 13=total number of infants in failure group & enrolled in the second intervention, 17=total number of infants in closure group, 12=total number of infants enrolled in the second intervention.

Discussion

The incidence of PDA in preterm infants is estimated to be 20 to 60%. Incidence rates are higher in small for gestational age and extremely low birth weight (ELBW) infants. In infants with birth weight below 1,200 grams, the incidence of PDA is approximately 80%. However, in those with birth weight below 2,500 grams, it is approximately 30%.18 In this study, we used birth weight criteria below 2,000 grams and gestational age below 36 weeks. Beyond gestational age and birth weight, the incidence of hemodynamically significant PDA was also dependent on diagnostic criteria based on clinical manifestations and echocardiography. Approximately 34% of PDA cases in preterm infants may close spontaneously at the chronological age of about 2 to 7 days, depending on the presence of hemodynamic disorders.[19]

Before the intervention, 10 subjects had neonatal asphyxia, 12 had respiratory distress syndrome (RDS), [19] had apnea of prematurity (AOP), 12 had thrombocytopenia, 12 had hyperbilirubinemia requiring phototherapy, and all subjects were suspected to have sepsis. We did not analyze the effect of these conditions on the success of the treatment, i.e., PDA closure. These conditions are considered complex problems for the management of preterm infants in tertiary hospitals in Indonesia. Nevertheless, subjects continued to receive supportive treatment based on clinical practice guidelines in our hospital.

We included 36 (28.3%) preterm infants with hs-PDA. Echocardiography examination was performed after the chronological age of about 72 hours. Although it is possible for PDA to close spontaneously in infants within the age range, we used the clinical hemodynamic consideration criteria for giving intravenous paracetamol. The incidence of PDA was difficult to precisely determine because not all preterm infants in our hospital undergo echocardiographic examination. Some infants died in the emergency room before this examination was done.

A systematic search of PubMed for previous studies on the use of paracetamol dosage in preterm infants yielded 13 studies consisting of 2 randomized clinical trials comparing oral paracetamol and oral ibuprofen,16,17 and 11 case series using oral or intravenous paracetamol.[5-15] For case series using intravenous paracetamol, the proportion of successful PDA closure was between 75 and 100%.12-15 Our design was similar, but we had a larger sample size compared to previous case series. The proportion of successful PDA closure was lower at 65.5%, than in the four previous case series.

Previous studies had varied indications for administering intravenous paracetamol. Oncel et al. used intravenous paracetamol for 10 cases who were contraindicated for oral therapy and had no history of COX-inhibitor use. PDA closure occurred in 7 cases after the first intervention, and in 3 cases after the second intervention.13 One child died, but no liver toxicity was found. However, Terrin et al. gave intravenous paracetamol to 8 cases with contraindication of COX-inhibitors. Six cases had PDA closure after the intervention.[14]

El-Kuffash et al. used intravenous paracetamol for 9 cases, consisting of 4 cases with ibuprofen treatment failure and 5 cases with contraindication of COX-inhibitors. After intervention, 5 cases had PDA closure and 3 cases had significantly decreased duct diameter. Two cases died, but no liver toxicity was found.[12] Moreover, in a study by Tekgunduz et al., intravenous paracetamol was given to 13 cases, consisting of 7 cases with contraindication for oral ibuprofen and 6 cases having side effects associated with oral ibuprofen administration. Ten cases had PDA closure, but 2 cases experienced reopening of the PDA.[15]

In this study, intravenous paracetamol was given to subjects who could not be given enteral nutrition. In addition, intravenous preparations of ibuprofen and indomethacin were not yet available in Indonesia. Twenty-nine (58.8%) preterm infants experienced closure of PDA after the first intervention, however, 4 cases died due to severe sepsis and multiple organ system failure. Four babies experienced closure of PDA after the second intervention. After the follow up at 14 days of life, 19 (64.5%) infants experienced PDA closure, and one case had reopening of the PDA.

In previous studies, the PDA diameter significantly decreased after intravenous paracetamol administration for 3 days.[12-15] We also found a significant difference of PDA diameter before and after the intervention. Based on this study and previous studies, intravenous paracetamol could be used as an alternative treatment for PDA closure in preterm infants, especially in cases contraindicated for ibuprofen and indomethacin. No significant difference in duct diameter was observed after the second

intervention and follow up at chronological age of 14 days. As such, we think that there would be no benefit for administering a third intervention in cases with treatment failure after the second intervention. Hence, if treatment failure persists after the second intervention, we suggest performing a PDA ligation.

As a result of the left-to-right shunt, hemodynamically significant PDAs in preterm infants increase the risk of comorbidities associated with prematurity.[1,18] During the study, we found one case of gastrointestinal bleeding and 3 cases of grade III/IV IVH, but our results do not explain the relationship between intervention with intravenous paracetamol and such comorbidities. We continued with the intervention because no contraindication for intravenous paracetamol existed. Although we did not examine plasma paracetamol levels, similar to previous studies we found no cases of liver toxicity or increased AST and ALT levels.

A limitation of this study was that the before-and-after design does not prove a causal relationship between the administration of intravenous paracetamol or other management and PDA closure. Subjects also received supportive therapy that might have affected the closing process of the PDA. We also cannot explain the mechanism of intravenous paracetamol in inhibiting PGE2 synthesis, as we did not measure plasma prostaglandin levels. Despite these limitations, a before-and-after study design is a practical choice for the evaluation of the effectiveness of a complex intervention, and it is commonly used in clinical practice when a randomized controlled trial is not feasible.

In conclusion, intravenous paracetamol is quite effective as an alternative treatment in the closure of PDA in preterm infants, especially in preterm infants with feeding intolerance or when oral therapy is contraindicated.

Conflict of interest

None declared.

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Original Article

Procalcitonin *vs.* combination of micro-erythrocyte sedimentation rate and C-reactive protein for diagnosing neonatal bacterial sepsis

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Abstract

Background Given the high rates of mortality and morbidity in neonatal sepsis, rapid, easy-to-use, and inexpensive biomarkers with high sensitivity and specificity are needed to diagnose neonatal sepsis. Procalcitonin is often used as a predictor in identifying neonatal sepsis, but C-reactive protein (CRP) and micro-erythrocte sedimentation rate (m-ESR) may also be valid biomarkers of neonatal sepsis.

Objective To compare the accuracy of procalcitonin to the combination of CRP and m-ESR, as well as to find cut-off points for the three tests, in diagnosing bacterial neonatal sepsis.

Methods Subjects were neonates hospitalized from July to October 2016 in Dr. Mohammad Hoesin Hospital, Palembang, South Sumatera, with sepsis at clinical presentation and healthy neonates with sepsis risk factors. All subjects underwent complete blood counts, CRP, m-ESR, blood cultures, and procalcitonin examinations.

Results Ninety-four infants were included, of whom 26 had proven sepsis. The combined values of m-ESR and CRP had 85% sensitivity, 59% specificity, and 66% accuracy. A procalcitonin (PCT) cut-off point of 9.7 ng/mL showed 100% sensitivity, 96% specificity, and 97% accuracy level, which were significantly higher than the combined values of m-ESR and CRP.

Conclusion The combined values of m-ESR (13 mm/hour) - CRP (17 mg/dL) and procalcitonin alone (2 ng/mL) are both valid for the diagnosis of bacterial neonatal sepsis, but the accuracy of procalcitonin at 9.7 ng/mL is significantly greater. **[Paediatr Indones. 2017;57:205-10 ; doi: http://dx.doi.org/10.14238/pi57.4.2017.205-10]**.

Keywords: *neonatal sepsis; m-ESR; CRP; procalcitonin; blood culture*

Neonatal sepsis is still the major health issue in some countries, especially in a developing country like Indonesia.[1,2] This condition leads to high rates of morbidity and mortality, especially in preterm and low birth weight babies.[3] Although neonatal intensive care unit (NICU) care has rapidly improved, the rate of mortality in sepsis is 20 to 50%.[1] Factors which influence the risk of infection are generally grouped into three categories: maternal, neonatal, and environmental.[1,2] Definitive diagnosis of neonatal sepsis is based on blood culture. Other supporting examinations to diagnose neonatal sepsis are white blood count (WBC), absolute neutrophil count (ANC), micro-erythrocyte sedimentation rate (m-ESR), and immature neutrophil /total neutrophil (I/T) ratio.[4-6] However, some of these examinations do not have high sensitivity and specificity for diagnosing neonatal sepsis. Additional examinations

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such as C-reactive protein (CRP) and more recently, procalcitonin (PCT), are expected to be more useful in diagnosing neonatal sepsis.[6]

Many studies about procalcitonin have shown that it had high sensitivity as a biomarker for neonatal sepsis.[7] Adib *et al.* reported a procalcitonin cut-off point of 1.1 ng/mL with sensitivity of 70%, specificity 80%, positive predictive value (PPV) 80%, and negative predictive value (NPV) 75%.[8] Also, Sucilathangam et al. reported that a procalcitonin cut-off point of ≥2 ng/mL had high sensitivity.[9] In addition, Chiesa *et al.* found that a 0.5-5 ng/mL procalcitonin cut-off point in neonatal bacterial sepsis had sensitivity 61-85% and specificity 50-97%.[10] Those doing procalcitonin research have yet to reach a consensus on a standard cut-off point for diagnosing bacterial neonatal sepsis.

In neonatal infectious diseases like sepsis and meningitis, CRP levels increase due to the local or systemic inflammatory response.[11] As such, CRP has been used as a parameter to establish a neonatal sepsis diagnosis. However, the studies have varied widely. Ng et al. reported that CRP examination had sensitivity of 84% and specificity of 96%, in 68 very low birth weight infants, as a single marker examination.[12] In contrast to other researchers, Anwer & Mustafa investigated fifty neonates with risk factors in pediatric intensive care unit of Abbasi Shaheed Hospital, Karachi Pakistan, were obtained that the CPR examination could help the establishment of neonatal sepsis diagnosis, CRP had the value of sensitivity of 60% and the specificity of 50%.[13] Another biomarker currently in use to diagnose neonatal sepsis is micro-ESR, an easy and inexpensive test to perform. Sharma *et al.* reported that micro-ESR had sensitivity 68.80%, specificity 76.50%, positive predictive value 57.90%, negative predictive value 83.90%, and accuracy and agreement of 74%.[14]

To improve sensitivity and specificity for diagnosing neonatal sepsis, combinations of biomarkers have been used. Philip *et al.* reported that two or more sepsis biomarkers such as I/T ratio ≥ 0.2, leukocytes < 5000/μL, positive CRP, positive haptoglobin, and mini-ESR ≥ 15/ in the first hour, had sensitivity of 93% and specificity of 88%, in infants with proven sepsis.[15] In addition, Mondal *et al.* used four sepsis biomarkers, namely, m-ESR (> 8 mm/1st hour), I/T ratio (>0.2), morphological changes in neutrophils, and CRP (≥ 6mg/L), and found that any positive two tests had sensitivity 84%, specificity 84%, and positive predictive accuracy 69%. Furthermore, for any three positive tests, sensitivity was 42%, specificity was 88%, and positive predictive accuracy was 95%. If four tests were positive, specificity and positive predictive values were 100%, but sensitivity was only 21%.[16]

Procalcitonin still has the highest sensitivity and specificity, yet it is expensive and has limited availability in Indonesian health centers. Therefore, we aimed to find a fast, easy to perform, and inexpensive screening tool, by combining the values of m-ESR and CRP, in the hopes of obtaining sensitivity and specificity values equivalent to those of procalcitonin.

Methods

This study was carried out in the Neonatal Intensive Care Unit (NICU) and Neonatal Ward of the Department of Pediatrics, Sriwijaya University Medical School/Dr. Mohammad Hoesin Hospital, Palembang, South Sumatera, from July to October 2016. Subjects were newborn infants with clinically diagnosed sepsis and healthy infants who had risk factors of neonatal sepsis, who had not yet received antibotics. Exclusion criteria were children who had perinatal asphyxia, hyaline membrane disease, necrotizing enterocolitis, or lack of parental consent.

Neonatal sepsis was defined as neonates with clinical presentation of sepsis and/or risk factors of neonatal sepsis, proven by blood culture (culture-proven sepsis). Major and minor criteria of suspected neonatal sepsis with the risk factors were as follows: major criteria- premature rupture of membranes > 18 hours, intrapartum fever > 38°C, chorioamnionitis, foul-smelling chorioamniotic fluid, and fetal heart rate > 160 times per minutes; minor criteria- premature rupture of the membranes > 12 hours, intrapartum fever > 37.5°C, low APGAR score, very low birth weight, gestational age < 37 weeks, twins, whitish vaginal discharge, and urinary tract infection.1 We used categories A and B clinical sepsis criteria,2 and the hematologic scoring system by Rodwell et al.17 as shown in **Table 1** and **Table 2**. Four predictors of neonatal sepsis were used, namely, m-ESR, CRP, the combination of m-ESR and CRP, and procalcitonin. The testing machine used, was from *i-Chroma* brand

(Korea) which used immunodetection method for CRP measurement.

Table 1. Hematologic scoring system for predilection of neonatal sepsis using Rodwell criteria[17]

Criteria	Abnormality	Score
Total WBC count	≤ 5,000/μL	1
	≥ 25,000 at birth	1
	≥ 30,000 at 12-24 hours	
	≥ 21,000 at day 2 in ward	
Total PMN count	No mature PMN soon	2
	Increased/decreased	1
Immature PMN count	Increased	1
I/T PMN ratio	Increased	1
I/M PMN ratio	≥ 0.3	1
Degenerative changes in PMN	Toxic granules/cytoplasmic vacuoles	1
Platelet count	≤ 150,000/μL	1

Table 2. Clinical findings in neonates with sepsis

A category	B category
• Respiratory distress (e.g., apnea, respiratory rates > 60 or <30 times per minute, costal retractions, expiratory grunting, central cyanosis)	• Tremor • Lethargy • Somnolence and • hypoactivity • Irritability
• Seizure	• Vomiting
• Decreased level of	• Abdomen distention
• consciousness	• Seen in the 4th day of live
• Abnormal body tem	• Mixed amniotic fluids
• perature	• Meconium-stained amniotic fluid
• Unhygienic delivery room	
• Rapid decrease and dramatically clinical presentation of the neonatal	• Poor sucking reflex

For the diagnostic evaluation of markers for bacterial neonatal sepsis, the sensitivity and specificity for each cut-off point for each marker were recorded. A comparison of the diagnostic accuracy of these markers was made by receiver-operating characteristic (ROC) curve analyses, by calculating the area under curve (AUC). Differences in accuracy were determined by comparing the confidence intervals of their accuracies. If the confidence intervals did not overlap, then the difference was considered to be significant. The estimation analysis with confidence intervals was used to compare the accuracy.

Results

During the study period, 577 neonates were admitted to the NICU and Neonatal Ward of Mohammad Hoesin Hospital. Ninety-four of them showed clinical sepsis or were healthy neonates with risk factors of neonatal sepsis, and were recruited into the study. Twenty-six (27%) neonates had positive blood cultures and were diagnosed to have bacterial neonatal sepsis. *Acinetobacter sp* and *Staphylococcus epidermidis* were the common causes of sepsis. **Table 3** shows the general characteristics of the study subjects. The ratio of males to females was 1.5:1. The majority of the ages of the subjects was < 72 hours (65 neonates, 69%), and at full term gestational age (54.2%). Also, the majority of subjects had weights > 2,500 grams (44 neonates, 46.8%).

As shown in **Table 4**, the combination of m-ESR (15 mm/hour) – CRP (10 mg/dL) had sensitivity of 85%, specificity 59%, and accuracy 66% (95%CI 55 to 75%). We used the reference value of procalcitonin ≥ 2ng/mL to diagnose bacterial neonatal sepsis, and found sensitivity to be 100%, specificity 68%, PPV 54%, NPV 100%, and accuracy value 77% (95%CI 67 to 84%).

In this study, we found different cut-off points of the three neonatal sepsis biomarkers, which we used singly or in combination. The cut-off points were m-ESR >13mm/hour, CRP >17 mg/dL, and procalcitonin 9.7 ng/m. For m-ESR of 13 mm/hour, the sensitivity was 85%, specificity 54%, PPV 42%, NPV 90%, and accuracy 63% (95%CI 52 to 72%) (data not shown). A CRP cut-off of 17mg/dL showed sensitivity of 88%, specificity 59%, PPV 45%, NPV 93%, and accuracy 67% (95%CI 56 to 76%). The combination of m-ESR 13mm/hour - CRP 17 mg/dL cut-off points yielded a sensitivity of 77%, specificity 72%, PPV 51%, NPV 89%, and accuracy 73% (95%CI 63 to 82%). Also, we found that PCT 9.7ng/mL had high sensitivity of 100%, specificity of 96%, and accuracy value of 97% (95%CI 90 to 99%). Hence, PCT was a significantly better biomarker for diagnosing bacterial neonatal sepsis. As shown in **Table 5**, PCT showed a greater accuracy compared with the combination of m-ESR-CRP.

Table 3. General characteristics of subjects

General characteristics	Bacterial neonatal sepsis			Range	
	Positive	Negative	Total	Minimum	Maximum
Age, n					
< 72 hours	17	48	65		
≥ 72 hours	9	20	29	1	10
Gender, n					
Male	15	41	56	-	-
Female	11	27	38		
Birth weight, n					
< 1,500 grams	1	6	7		
1,500-2,500 grams	11	32	43	1,150	4,000
> 2,500 gram	14	30	44		
Gestational age, n					
Pre-term	11	32	43	29 weeks	40 weeks
Full term	15	36	51		

Table 4. Laboratory markers

Laboratory markers	Bacterial neonatal sepsis			Range	
	Positive	Negative	Total	Minimum	Maximum
m-ESR, n					
> 15 mm/hour	18	27	45	2.0	90
≤ 15 mm/hour	8	41	49		
CRP, n					
> 10 mg/L	26	42	69	5.0	275
≤ 10 mg/L	0	26	26		
PCT, n					
≥ 2 ng/mL	26	22	48	0.16	100
< 2 ng/mL	0	46	46		

Table 5. The comparison of four predictors in diagnosing bacterial neonatal sepsis

Cut off points of biomarkers	Sens, %	Spec, %	PPV, %	NPV, %	Accuracy, %	95%CI of accuracy
CRP > 10 mg/dL + m-ESR ≥ 15 mm/hour	85	59	44	77	66	55 to 75
PCT ≥ 2 ng/mL	100	68	54	100	77	67 to 84
PCT ≥ 9.7 ng/mL	100	96	90	100	97	90 to 99
CRP > 17 mg/dL + m-ESR > 13 mm/hour	77	72	51	89	73	62 to 82

Discussion

Neonatal sepsis is a common and catastrophic illness.[18] We aimed to compare the combination of some sepsis biomarkers in order to find an early and accurate means of diagnosis to decrease morbidity for neonatal sepsis. Our study provides insight into the diagnostic value of the combination CRP and m-ESR in neonatal bacterial sepsis. Using m-ESR of 15 mm/hour and CRP 10mg/dL, a fairly high 85% sensitivity value was obtained, but the specificity was only 59%, with PPV 44%, NPV 77%, and accuracy value of 66%. Similarly, Mondal *et al.* used four sepsis biomarkers [m-ESR (>8mm/1st hour), I/T ratio (>0.2), morphological changes in neutrophils, and CRP (≥6mg/L)] and found that by combining two, three, or even four sepsis biomarkers, the sensitivity was 84%, specificity 84%, and PPV was 69%.[16]

Statistical significance was obtained with comparison of the value of accuracy between the combination of value of accuracy of m-ESR was ≥ 15 mm/hour and CRP was > 10 mg/dL with the value of accuracy of procalcitonin by the cut off point was ≥ 2 ng/mL. Yet, if we compared to the procalcitonin with the cut off point was ≥ 9.7 ng/dL, it was obtained that the value of accuracy was not significant or different from the value of accuracy of the procalcitonin.

Using new cut-off points of m-ESR > 13 mm/hour and CRP > 17 mg/dL, we obtained the values of sensitivity and specificity, 77% and 72%, respectively, and a higher value of accuracy of 73% compared to the combined value of m-ESR 15mm/hour and CRP 10 mg/dL, which had an accuracy of 66%. Hence, the new cut-off point was more accurate for diagnosing neonatal sepsis. Philip *et al.* also found that using two or more sepsis biomarkers such as I/T ratio ≥ 0.2, leukocytes < 5,000/μL, positive CRP, positive haptoglobin, mini-ESR ≥ 15 mm in the first hour, resulted in the higher sensitivity and specificity values.[15] A comparison of the new cut-off point of m-ESR and CRP values with the procalcitonin reference value of > 2 ng/mL, revealed similar values in the level of accuracy. Yet, when we used the PCTcut-off of 9.7ng/mL, accuracy dramatically improved. This finding may have been due to procalcitonin value of 9.7 ng/mL having a high value for diagnosing bacterial neonatal sepsis, compared with other markers. Esmat *et al.* also showed that PCT with a 4ng/mL cut-off had sensitivity 100%, specificity 50%, PPV 44.4%, and NPV 100%.[19]

Sensitivity, specificity, and accuracy in diagnosing bacterial neonatal sepsis level is good when we used a PCT cut-off point of 9.7 ng/mL by itself. A previous study has shown that PCT levels were markedly higher in patients with bacterial sepsis than in healthy controls,[20] and PCT was found to be superior to other biomarkers, especially CRP.[21] In a head-to-head comparison with CRP, PCT rose faster (4 hours compared to 6 hours in CRP), peaked faster (8 hours compared to 36-50 hours), normalized faster (48 hours after appropriate therapy compared to 72-96 hours), and was more sensitive and specific to sepsis (92.6% and 97.5%, respectively).[22]

In conclusion, the combination of micro-ESR 13 mm/hour and CRP 17 mg/dL can be used to diagnose bacterial neonatal sepsis, but PCT level of 9.7 ng/mL is a better measurement to diagnose bacterial neonatal sepsis, with sensitivity of 100% and specificity 96%.

Conflict of Interest

None declared.

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Original Article

Irrational use of antibiotics and clinical outcomes in children with pneumonia

Yusuf, Indah Kartika Murni, Amalia Setyati

Abstract

Background Pneumonia is a major cause of morbidity and mortality in children under five. Antibiotic treatment must be started immediately in children with pneumonia. The irrational use of antibiotics may increase morbidity and mortality in children with pneumonia.

Obejctive To determine the prevalence of the irrational use of antibiotics and clinical outcomes in children with pneumonia.

Methods We conducted a cross-sectional study in children with pneumonia who were admitted to the Pediatric Ward or PICU at Dr. Sardjito Hospital, Yogyakarta, from December 2010 to February 2013. Data were obtained from subjects' medical records. Children with malnutrition, congenital heart defects, sepsis, shock, central nervous system disorders, syndromes, or other concomitant infections were excluded.

Results Of 46 children who fulfilled the inclusion criteria, 13 (28.3%) used antibiotics irrationally and 7 (15.2%) died. Most subjects were aged less than 1 year (25 subjects, 54.3%) and 1 - < 5 years (18 subjects, 39.1%). The female to male ratio was 1:1. Most cases were referred from other hospitals (23 subjects, 50%). Twenty-eight (60.9%) subjects stayed in hospital > 7 days. Ampicillin was the most common first-line, empirical antibiotic used (32 subjects, 69.6%). Blood cultures were obtained in 20 (43.5%) patients, yielding no growth in 16 subjects, coagulase-negative staphylococci (CONS) in 3 subjects, and *Pseudomonas aeruginosa* in 1 subject. The irrational use of antibiotics was significantly associated with mortality in a univariate analysis [PR 6.35; (95%CI 1.40 to 28.69); P=0.006].

Conclusion The irrational use of antibiotics is common among children with pneumonia and is significantly associated with mortality. **[Paediatr Indones. 2017;57:211-5 ; doi: http://dx.doi.org/10.14238/pi57.4.2017.211-5]**.

Keywords: *antibiotic; irrational; pneumonia*

Bacterial pneumonia is the main cause of morbidity and mortality in children below 5 years of age. The incidence and its mortality are higher in developing countries. The incidence of bacterial pneumonia in children below 5 years of age was estimated to be 0.29 episodes each year for children in developing countries, and 0.05 episodes for children in developed countries.[1] In 2013, there were 156 million new episodes for the year worldwide, with as many as 151 million in developing countries. Most cases were found in India (43 million), China (21 million), Pakistan (10 million), as well as Bangladesh, Indonesia, and Nigeria (6 million each).[2]

Antibiotic therapy must be started immediately in children with suspected community-acquired pneumonia (CAP) caused by bacteria.[3] Inappropriate antibiotic treatment may lead to greater expense, toxic side effects, antibiotic resistance, and superinfections that are difficult to treat. Thus, antibiotics should be

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used rationally for the treatment of pneumonia.[4,5] Irrational use of antibiotics significantly increased morbidity and mortality in children with infections, including pneumonia.[6] Although antibiotics have an important role in reducing mortality of pneumonia, research has been limited. We aimed to evaluate the irrational use of antibiotics and outcomes in children with pneumonia at Sardjito Hospital.

Methods

This cross-sectional study was conducted in the Pediatric Ward and PICU of Sardjito Hospital, Yogyakarta, Central Java, in November 2016. Subjects were children with pneumonia treated according to standard medical procedures of Sardjito Hospital, aged 1 month - < 18 years, and hospitalized between December 2010 and February 2013. Data were obtained from patients' medical records. The exclusion criteria were children with malnutrition, congenital heart defects, sepsis, shock, central nervous system disorders, disease syndromes, or other concomitant infections. The minimum required number of subjects was calculated to be 46, with 0.05 confidence level and 80% power.

The subjects' basic characteristics were age, sex, and case classification. The outcomes were classified into primary (survived/died) and secondary (length of hospital stay, rational/irrational use of antibiotics, type of antibiotics used, first-line antibiotics, combination antibiotics, blood cultures, and types of microorganisms). The independent variable was the irrational use of antibiotics, while the dependent variables were primary outcomes (survived or died) and the secondary outcomes of hospital length of stay of 0-7 days or > 7 days.

Pneumonia was a diagnosis made by treating clinicians and was written in medical records as a final diagnosis based on ICD 10 of pneumonia which was J18. The diagnosis of pneumonia in Dr. Sardjito Hospital was made by clinical and radiological findings. The type of the pneumonia in this study was CAP. The irrational use of antibiotics was defined as antibiotic use that was not in accordance with that recommended for particular indications, dose, and/or length of treatment. Length of stay was defined to be the number of days hospitalization from the time of admission to the time of discharge, regardless of primary outcome and classified into two categories: 0-7 days or > 7 days. Type of case was classified as community, if patients had come directly to the hospital, referral, if patients were referred by another hospital, or transfer, if patients were initially treated in the PICU.

Data was described and analyzed with P values, prevalence ratio (PR), 95% confidence intervals. Bivariate analysis was done with Chi-square and Fisher's tests. Statistical analysis was performed with *SPSS* software. This study was approved by the Ethics Committee for Medical Research, Gadjah Mada University Medical School.

Results

Fourty six children were fulfilled the inclusion criteria, of whom 13 (28.3%) received irrational use of antibiotics and 7 (15.2%) died. The characteristics of subjects are shown in **Table 1**.

Table 1. Characteristics of subjects

Characteristics	N=46
Gender, n(%)	
Male	23 (50.0)
Female	23 (50.0)
Age, n(%)	
< 1 year	25 (54.3)
1 - < 5 years	18 (39.1)
5 - < 10 years	1 (2.2)
≥ 10 years	2 (4.3)
Type of case, n(%)	
Community	21 (45.7)
Referral	23 (50.0)
PICU transfer	2 (4.3)

Data on primary outcomes, rationality of antibiotics, type of irrational use, length of stay, blood culture results, types of microorganisms, and empirical therapy are shown in **Table 2**.

Univariate analysis of primary outcomes and rationality of antibiotic use is shown in **Table 3**. Univariate analysis of length of stay and rationality of antibiotic use is shown in **Table 4**.

Table 2. Clinical outcomes

Outcomes	N=46
Primary outcomes, n(%)	
Survived	39 (84.8)
Died	7 (15.2)
Rationality of antibiotic use, n(%)	
Irrational	13 (28.3)
Spectrum/indication	12 (92.3)
Length of treatment	1 (7.0)
Dose	0
Rational	33 (7.17)
Length of stay, n(%)	
0-7 days	18 (39.1)
> 7 days	28 (60.9)
Blood culture, n(%)	
Culture	20 (43.5)
No growth	16
Coagulase-negative staphylococcus	3
(CONS)	1
Pseudomonas aeruginosa	26 (56.5)
No culture	
First-line antibiotics, n(%)	
Ampicillin	32 (69.6)
Ceftriaxone	5 (10.9)
Cefotaxime	8 (17.4)
Imipenem	1 (2.2)
Antibiotics combination, n(%)	
Cefotaxime	1 (3.3)
Ceftazidime	1 (3.3)
Cefixime	1 (3.3)
Chloramphenicol	14 (46.7)
Amikacin	2 (6.7)
Gentamicin	11 (36.7)

Note: Antibiotics combination were given in 30 patients

Table 3. Univariate analysis of primary outcomes and rationality of antibiotic use

Antibiotic use	Primary outcomes		PR (95%CI)	P value
	Died	Survived		
Irrational	5	8	6.35 (1.40 to 28.69)	0.006
Rational	2	31		

Table 4. Univariate analysis of length of stay and rationality of antibiotic use

Antibiotic use	Length of stay		PR (95%CI)	P value
	More than 7 days	0-7 days		
Irrational	10	3	1.41 (0.92 to 2.17)	0.161
Rational	2	31		

Discussion

Of 46 children with pneumonia, 7 (15.2%) died, similar to that reported by Latumahina *et al.* (15%).1 Thirteen of our subjects (28.3%) received irrational antibiotic treatment for pneumonia. A previous study reported a similar 24% in Sardjito Hospital, although higher percentages were reported in Mongolia (56.6%), Turkey (56.5%), and India (56%). These differences may have been due to studying only children below 5 years of age or including adults.[4,7,8,9]

Most of our subjects were < 1 year of age (25; 54.3%) or 1 - < 5 years old (18; 39.1%), with a 1:1 ratio of males to females. Pneumonia was found to be the main cause of morbidity and mortality in children below 5 years by Latumahina *et al.* and the Indonesian Ministry of Health.[1,10]

Most pneumonia was caused by infectious agents, although non-infectious causes included food or gastric acid aspiration, foreign bodies, hydrocarbon and lipoid agents, hypersensitivity reactions, drugs, and radiation pneumonitis. Most of the time it was hard to find the cause of pneumonia, because invasive specimen collection was rarely, if ever, performed. Specimens from the upper respiratory tract or sputum are usually not accurate for determining the cause of lower respiratory tract disease.[11]

Treatment of pneumonia is based on the causative agent and clinical findings,[12] although, generally, clinical signs do not help to differentiate etiologies of pneumonia. Early identification of etiology is also difficult, so antibiotics are usually chosen by an empirical approach. All patients in this study received empirical antibiotic treatment. Ampicillin was the most common first-line, empirical antibiotic used (32; 69.6%), while the most common combination antibiotics used were chloramphenicol (14; 46.7%) and gentamicin (11; 36.7%). These findings were in agreement with the *World Health Organization* (WHO) and the Indonesian Pediatrics Society (IPS) recommendations that children with severe and very severe pneumonia be hospitalized, receive ampicillin as the first-line treatment, and be observed for next 24 to 72 hours. If the patient has a good response, treatment must be continue for 5 days. But if the patient becomes worse within 48 hours or experiences severe clinical conditions (unable to eat/drink, vomiting at all feedings, seizure,

lethargy, unconsciousness, or cyanosis with respiratory distress), chloramphenicol must be added. Patients with severe clinical conditions should directly be given a combination of ampicillin-chloramphenicol or ampicillin-gentamicin. If the patient is unresponsive to the above antibiotics, amikacin or cephalosporin could be used.[13]

We noted that empirical therapy was not always given according to the recommended protocol, such as with the use of cefotaxime, imipenem, and ceftazidime. This finding may have been due to most cases (23; 50%) being referred from other hospitals or directly treated in the PICU (2; 4.3%). The antibiotics used in other hospitals also might have influenced the sensitivity of microorganisms to antibiotics, as increased antibiotic resistance among respiratory infectious agents might affect the choice of empirical treatment.[14]

From 13 subjects who used antibiotics irrationally, almost all were considered irrational based on spectrum of disease (12; 92.3%), while only 1 (7.7%) was based on duration. There was no irrational use based on dosage. However, these results were not subjected to a qualitative antibiotic evaluation using Gyssens pathway, that classifies antibiotic use into six categories: I. incorrect usage, IIa. incorrect dose, IIb. incorrect intervals, IIc. incorrect route, IIIa. incorrect due to long duration, IIIb. incorrect due to short duration, IVa. incorrect due to a more effective antibiotic, IVb. incorrect due to a closer spectrum, V. no indication for antibiotics, and VI. medical records not complete enough to evaluate. The antibiotic is correct if the evaluation matches with category I, but incorrect if it is IIa, IIb, IIc, IIIa, IIIb, IVa, IVb, IVc, IVd, V (II,III,IV,V).[15]

In developing countries, hospital microbiology data of that could be used to guide patient management is rare to non-existent. Specimen collection from the lungs to assess the pneumonia etiology is not possible, so specimens were taken from tracheal aspiration of intubated patients. This specimen is not sensitive enough to define the pneumonia etiology.[11] In addition, blood cultures from pneumonia patients are not routinely performed, except in cases of very severe pneumonia. In our study, 20 subjects had cultures started on the day of admission, but only 4/20 had positive findings: 3/20 coagulase-negative staphylococcus (CONS) and 1/20 Pseudomonas aeruginosa. This result differed from the reported pneumonia etiologies in children of Streptococcus pneumoniae, Haemophilus influenza, and respiratory syncytial virus.[16,17] Blood cultures in PICU patients at Cipto Mangunkusumo Hospital (CMH), Jakarta, were similar to our findings at Sardjito Hospital, as those CMH patients had mostly Pseudomonas (33.1%), coagulase-negative staphylococcus (19.5%), and Klebsiella pneumoniae (13.3%).[11] The most commonly found species in this study was coagulase-negative staphylococcus (15%), but blood culture was performed in only 20 patients, 4 of which grew bacteria. As such, these findings are too weak to be the basis of microorganism sensitivity data.

It has been shown that adequate antibiotic treatment shortens the length of stay and decreases mortality. The dilemma is that decreased antibiotic usage decreases resistance, but delayed or inadequate treatment increases the mortality and morbidity of pneumonia, especially that caused by Gram-negative bacteria.[18] We also found a significant association between irrational use of antibiotics and death (PR 6.35; 95%CI 1.40 to 28.69; P=0.006).

Antibiotic resistance and death outcome could not be analyzed because only 20 subjects underwent blood cultures. Those patients were the severe or very severe cases, so they did not reflect the general study population.

Athale et al. reported that inadequate empirical antibiotics for 30 days length of stay resulted in higher mortality (11.1%) than did a 7-day length of stay (3.7%). For delayed empirical therapy, there was no significant difference in mortality between 7 days or 30 days length of stay.[19]

We used a 7-day limit for the length of stay outcome based on the standard duration of pneumonia treatment of 5-7 days.[13] Eighteen subjects (39.1%) had a 0-7-day length of stay; 28 subjects (60.9%) had a > 7 day length of stay. No significant association was observed between irrational use of antibiotics and length of stay, indicating that the severity of the disease could influence the length of stay.

This study has several limitations. First, we used retrospective data, so risk factors and outcome were taken from one point of time. Second, there was no precise data on the severity of pneumonia or previous history of hospitalization, such as length of stay or previous antibiotics used. Third, a qualitative

evaluation of antibiotic treatment was done with only 3 parameters: incorrect spectrum/indication, dose, and length of treatment, so the classification of irrational antibiotic use was weak.

In conclusion, irrational use of antibiotics in children with pneumonia at Dr. Sardjito Hospital is significantly associated with death, but we find no such relationship with length of stay. Irrational use of antibiotics was defined by simple clinical and laboratory data that were used to diagnose pneumonia. Further study using Gyssens pathway, the degree of pneumonia severity, previous hospitalization history (length of stay and antibiotic use), blood cultures for all subjects, and better study methods are needed.

Conflict of Interest

None declared.

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Original Article

Quality of life among obese and non-obese early adolescents

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Abstract

Background Obesity in adolescents adversely affects both their psychological as well as their physical health.

Objective To compare the quality of life between obese and non-obese early adolescents, using the PedsQL inventory.

Methods A cross-sectional study was carried out on early adolescents aged 10-12 years among several elementary schools in Denpasar, Bali. Body mass index (BMI) percentiles for age and sex were categorized as obese (BMI $\geq 95^{th}$ percentile) and non-obese (BMI $<95^{th}$ percentile). Data on quality of life were collected using *PedsQL Generic Core Scales version 4.0* inventory, filled by the children and their parents separately.

Results Total PedsQL score in obese and non-obese group were significantly difference in both reports [child report: mean difference of 9.59 (95%CI 7.14 to 12.05; P<0.05) and parent-proxy report: mean difference at 8.95(95%CI6.64 to 11.26; P<0.05)]. After classifying subjects into impaired and not impaired quality of life based on a total score cut-off <78 as well as other cut-off points for each domain, the individual domains of physical, social, and school function were also significantly associated with obesity (child report: P=0.02, P< 0.001, P=0.018, respectively, and parent-proxy report:P=0.007, P<0.001, P<0.001, respectively). However, emotional function was not significantly associated with obesity (P>0.05). After adjusting for age, gender, and parental education, obesity was significantly associated with PedsQL scores in the child report (OR 7.25; 95%CI 2.94 to 17.89; P<0.05) and the parent-proxy report (OR 10.87; 95%CI 3.83 to 30.84; P<0.05).

Conclusion Obese early adolescents report significantly poorer quality of life with regards to the physical, social, school function domains and total quality of life than those who are classified into non-obese. **[Paediatr Indones. 2017;57:216-22 ; doi: http://dx.doi.org/10.14238/pi57.4.2017.216-22]**.

Keywords: *obese; early adolescent; health-related quality of life; PedsQL*

Obesity is a serious public health problem in children and adolescents, and is an early risk factor for many adult morbidity and mortality issues. Overweight and obesity are reported to be associated with an increased risk of hypertension, coronary arterio sclerosis, elevated cholesterol, type 2 diabetes, joint problems, stroke, and certain types of cancers. Health consequences of overweight and obesity are not just limited to physical health; overweight and obese children experience problems including body dissatisfaction, negative body image, low self-esteem, anxiety, depression, stigmatization, and social marginalization, all of which can influence their psychological and social health.[1,2]

The prevalence of overweight and obesity in children and adolescents has risen in both developed and developing countries in recent decades. *Riset Kesehatan Dasar (Riskesdas)* in 2013, reported that

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the prevalences of obesity in children under five in 2007, 2010, and 2013, was 12.2%, 14.0% and 11.9%, respectively. In addition,the obesity prevalences in children aged 5-12, 13-15, and 16-18 years was 8.8%, 2.5%, and 1.6%, respectively.[3] In the city of Denpasar, the prevalence of obesity in elementary school children was 15%.[4]

Quality of life of obese children and adolescents should receive more attention because of the impact of obesity itself. The WHO define quality of life as "the individual's perception of their position in life in the context of the culture and value systems in which they live, and in relation to their goals, expectations, standards, and concerns," in other words, a global view that considers many dimensions of human beings. Measures of quality of life assess important aspects of health, included the effect of a health condition on the child's daily activities, physical symptoms, social interactions, and emotional wellbeing.[5]

Studies have shown a consistent relationship between abnormal weight and the perception of low quality of life in children and adolescents.3 Khodaverdi et al. in 2011 found that the quality of life of obese children in physical function, social function, and school function were significantly lower than children with normal weight.[6] Furthermore, in early adolescent (10-14 years) some changes can occur, such as anxiety of body appearance, hormonal changes affect the emotion, and navigating friendships and groups of friends. Adjustment to their environment may pose problems for early adolescents, as they leave childhood and enter a new phase that full of challenges. Such adjustments can certainly influence their quality of life.[7]

Information about obesity and quality of life in early adolescents in Denpasar, Bali, was limited. The aim of this study was to evaluate the association between health-related quality of life and weight status in a community sample of school children aged 10–12 years in Denpasar, Bali.

Methods

A cross-sectional study was carried out by children among five public elementary schools in Denpasar, Bali,who were selected via consecutive sampling. This study used a significance level of $P<0.05$ and power of 80%, resulting in sample size of at least 48 for each group. The inclusion criteria were: children aged 10 to 12 years (of both sexes), parents willing to participate in the study and agreed the informed consent. The exclusion criteria were: children with severe sensory and communication disability (e.g., blindness or deafness) or severe motor disability (e.g., cerebral palsy or hydrochepalus), chronic disease (e.g., asthma, diabetes, malignancy, heart disease), underweight (BMI<5th percentile), incomplete questionnaires.

The PedsQL 4.0 was used to assess health-related quality of life in this study. This inventory was a validated, 23-item questionnaire for children aged 2–18 years, administered as either a child self-report or a parent proxy-report.[8] *PedsQL Generic Core Scales version 4.0* inventory was used for children and adolescents aged 8 to 12 years. In brief, the PedsQL comprised four subscales: physical (8 items), social (5 items), emotional (5 items), and school function (5 items). The instructions asked how much of a problem each item had been during the last month. A five-point response scale was used (0=never, 1=almost never, 2=sometimes, 3=often, 4=always). Items were reverse-scored and linearly transformed to a 0–100 scale (0=100, 1=75, 2=50, 3=25, 4=0), so that higher scores indicated better quality of life. A total scale score, derived by the mean of all 23 items, was calculated to provide an overall measure of the quality of life.[8]

All participating children had anthropometric measurements taken in school by trained research staff using standardized procedures and equipment. Height was measured to the nearest 0.5 cm using a portable stadiometer. Weight was measured to the nearest 0.1 kg using scales. BMI was calculated as weight (kg)/height (m^2) and percentiles for age and sex were categorized into obese (BMI $\geq$ 95th percentile) or non-obese (BMI $<$ 95th percentile). Parent's education was divided into high and low. Parent who had completed senior high school were classified into high education category.

Total PedsQL score and domain score in child and parent-proxy report were grouped by different cut-off, and used to identify special health care need as well as children who may have needed services above what is normally expected. The cut-off for each domain were used to define the categories of impaired and not impaired quality of life. The cut-off

scores were as follows physical function ≤88 and >88, emotional function ≤75 and >75, social function ≤75 and >75, school function ≤70 and >70, and total score ≤78 and >78.[9]

The Ethics Committee of Udayana University Medical School approved the study. Written informed consent was obtained from parents of the participating children and oral consent obtained from children. Furthermore, the children and their parents also were informed that they had the right to withdraw from the study at any time and were assured of confidentiality.

All statistical analyses were performed using SPSS for Windows. Descriptive statistics were used to summarize demographic and anthropometric data. The independent sample T-test (two groups) was used to analyze differences in PedsQL scores between groups, while Chi-square test was used to analyze the relationship between obesity and PedsQL scores.

Results

Written informed consent was sought from parents of 310 eligible children aged 10 to 12 years, 230 (74.19%) of those who agreed to participate. After exclusion of 97 student questionnaires, which were incomplete, the remaining number of subject was 133. The characteristics of subjects are shown in **Table 1**. Fifty children were obese (group 1) and 83 were

Table 1. Subjects' characteristics (N=133)

Characteristics	Obese (n=50)	Non-obese (n=83)
Median age (range), years	11 (10-12)	11 (10-120
Gender, n(%)		
Male	29 (58)	42 (50.6)
Female	21 (42)	41 (49.4)
Median age of parents (range), years		
Father	41 (34-54)	41 (33-57)
Mother	39 (32-53)	38 (31-58)
Maternal education, n(%)		
Low	4 (8)	13 (15.7)
High	46 (92)	70 (84.3)
Paternal education, n(%)		
Low	8 (16)	10 (12)
High	42 (84)	73 (88)
Median anthropometric measures (range)		
Weight, kg	53.00 (39-70)	41 (32.52)
Height, cm	144 (132-160)	148 (135-136)
BMI, kg/m^2	25.38 (22.38-32.09)	19.17 (14.28-23.78)
Median PedsQL score (range)		
Physical function		
Child report	73.44 (43.75-90.62)	82.50 (59.38-96.88)
Parent-proxy report	75.00 (59.38-90.62)	84.37 (59.38-100)
Physical function		
Child report	75.00 (30.00-95.00)	75.00 (60.00-90.00)
Parent-proxy report	75.00 (35.00-90.00)	80.00 (55.00-95.00)
Physical function		
Child report	75.00 (50.00-90.00)	90.00 (55.00-100)
Parent-proxy report	75.00 (50.00-90.00)	90.00 (50.00-100)
Physical function		
Child report	70.00 (45.00-90.00)	75.00 (50.00-90.00)
Parent-proxy report	70.00 (50.00-80.78)	75.00 (50.00-90.00)
Mean PedsQL score (SD)		
Child report	70.63 (8.31)	80.23 (5.92)
Parent-proxy report	71.98 (7.05)	80.93 (6.19)

non-obese (group 2). In obese group, there were 29 (58%) boys and 21 (42%) girls, with median age of 11 (range 10 to 12) years. In non-obese group, there were 42 (50.6%) boys and 41 (49.4%) girls, with median age of 11 (range 10 to 12) years. The median age of parents (fathers and mothers) in the obese group were 41 and 39 years, respectively, and 41 and 38 years, respectively, in the non-obese group. In both groups, most fathers and mothers were highly educated. All families in both groups had an average monthly income above the regional minimum wage.

The median weight, height, and BMI in the obese group were 53.00 (range 39-70) kg, 144 (range 132-160) cm, and 25.38 (range 22.38 to 32.09) kg/m^2, respectively. For non-obese group, the median were 41 (range 32 to 52) kg, 148 (range 135-156) cm, and 19.17 (range 14.28 to 23.78) kg/m^2, respectively.

Median score reported for each domain (physical, social, and school function) in obese group compared to non-obese group were 73.44 *vs.* 82.55, 75.00 *vs.* 90.00, and 70.00 *vs.* 75.00, respectively. The emotional function child report scores were similar in both groups (median 75.00). Median scores of the parent-proxy report for each domain function (physical, emotional, social, and school function) in the obese compared to non-obese group were 75.00 *vs.* 84.37, 75.00 *vs.* 80.00, 70.00 *vs.* 90.00, and 70.00 *vs.* 75.00, respectively. The mean total PedsQL scores in the obese compared to the non-obese group were 70.63 (SD 8.31) *vs.* 80.23 (SD 5.92) with mean difference 9.59 (95%CI 7.14 to 12.05; P<0.05) for the child report, and 71.98 (SD 7.05) *vs.* 80.93 (SD 6.19), with mean difference 8.95 (95%CI 6.64 to 11.26; P<0.05) for the parent-proxy report. The total PedsQL score

Table 2. Association of nutritional status and PedsQL scores according to child self-report

Variables	Quality of life	Nutritional status				OR	95%CI	P value
		Obese		Non-obese				
		n	%	n	%			
Physical function	Impaired	46	92	58	69.9	4.957	1.61 to 15.25	0.02
	Not impaired	4	8.0	25	30.1			
Emotional function	Impaired	34	68.0	46	55.4	1.71	0.82 to 3.57	0.105
	Not impaired	16	32.0	37	44.6			
Social function	Impaired	29	58.0	8	9.6	12.95	5.16 to 32.49	<0.001
	Not impaired	21	42.0	75	90.4			
School function	Impaired	30	60.0	33	39.8	2.27	1.11 to 4.65	0.018
	Not impaired	20	40	50	60.2			
Total score	Impaired	42	84.0	37	44.6	6.53	2.73 to 15.59	<0.001
	Not impaired	8	16.0	46	55.4			

Table 3. Association of nutritional status and PedsQL scores according to parent-proxy report

Variables	Quality of life	Nutritional status				OR	95%CI	P value
		Obese		Non-obese				
		n	%	n	%			
Physical function	Impaired	46	92.0	61	73.5	4.15	1.34 to 12.87	0.007
	Not impaired	4	8.0	22	26.5			
Emotional function	Impaired	29	58.0	41	49.4	1.42	0.69 to 2.87	0.105
	Not impaired	21	42.0	42	50.6			
Social function	Impaired	31	62.0	3	3.6	43.51	12.02 to 157.48	<0.001
	Not impaired	19	38.0	80	96.4			
School function	Impaired	41	82.0	35	42.2	6.25	2.69 to 14.51	0.018
	Not impaired	9	18.0	48	57.8			
Total score	Impaired	43	86.0	36	43.4	8.02	3.23 to 19.91	<0.001
	Not impaired	7	14.0	47	56.6			

and scores for each domain function in the child and parents-proxy reported classified as impaired or not impaired quality of life, by different cut-off. **Table 2** and **Table 3** show the association between obesity and total PedsQL scores.

After age, gender, and parental education adjusted, obesity was significantly associated with impaired quality of life in both child and parent-proxy report (OR 7.25; 95%CI 2.94 to 17.89; P<0.05 and OR 10.87; 95%CI 3.83 to 30.84; P<0.05, respectively).

Discussion

In our study, health-related quality of life scores were measured using PedsQL Generic Core Scales version 4.0 inventory. In repeated reliability and validity tests, the PedsQL has consistently had high reliability scores (α= 0.71-0.89) and was also able to distinguish between healthy children and those with chronic diseases.[10] There is no definitive cut-off point to determine the value of a good or a poor quality of life on children and adolescents. Huang et al. reported that the recommended cut-off scores for children <8 years were 83, 79 for moderate, and 77 for major chronic conditions. For children ≥ 8 years, the cut-off scores were 78, 76, and 70, respectively.[9] Khairy *et al.* also developed a classification of PedsQL score. They calculated total score from the four domain scores, out of a possible 100, then changed the total score into percentage and grouped the percentages. Scores <25% were interpreted as bad quality of life, 25% to <50% as fair quality of life, 50 to <75% as good quality of life, and 75–100% as very good quality of life.[1] In our study, we followed the classification of Huang et al. for children ≥ 8 years of age.

Obese early adolescent reported poorer quality of life in the physical, social, and school function domains, as well as in total quality of life than non-obese early adolescents. This finding suggests that obesity has a negative impact on children and adolescent's daily life. Similarly, Riazi *et al.* in the United Kingdom stated that obese and overweight groups reported impairment in all quality of life dimensions compared to the normal weight group.[11] However, Hughes et al. in the United Kingdom, found that only physical health was significantly impaired in obese children aged 8 to 12 years.[12] In our study, both child and parent-proxy reports showed lower scores for each domain function in the obese group compared to the non-obese group. The mean total PedsQL score was also significantly lower in the obese group compared to the non-obese group. After classification into impaired vs not impaired quality of life based on different cut-off points for each domain, only the emotional function was not significantly associated with obesity. Children aged 10 to 12 years are included in early adolescence. At that age the child's emotional maturity function is influenced by many factors. Individual factors, such as cognitive development and temperament, influence the development of emotional competencies. These skills are also influenced by past social experience and learning, including an individual's relationship history, as well as the system of beliefs and values in the which the person lives.[14]

The total quality of life scores were evaluated for relationships to characteristics of the obese and normal children. Recent study found a significant, negative correlation between total quality of life scores and BMI, waist circumference, and weight. Their observation indicates that increased BMI, waist circumference, and weight leads to impairment and negative impacts on the quality of life of children.1 An explanation is that excess weight may lead to a decrease in their physical functional health status. These findings are consistent with that of Abdel Aziz et al. in Egypt, who found that overweight children had significantly worse physical function than children with normal BMI.[13]

Obese children and adolescents were more likely to experience psychosocial problems than their normal weight peers. Obesity stigma, teasing, and bullying are pervasive, and can have serious consequences for emotional and physical health, and school performance. A previous study of depression and adolescent obesity showed that weight gain during adolescence may be related to depression, negative mood status, and poor self-esteem.[15] Obese children, compared with normal weight children, were found to be significantly more likely to experience depression.[16] Recent study findings confirmed that obese adolescent had significantly lower self esteem than normal weight peers, as measured by various focused questionnaires. Findings confirmed that a clear negative impact on self-esteem was associated with obes adolescent.[17]

We found that school function scores were significantly lower in the obese than in the non-obese group, in the child and parent-proxy reports. These findings are consistent with a Hong Kong study, in which school function of normal weight children was significantly higher than the group of overweight children.[18] In contrast, Williams *et al.* in Australia found that the difference in school function scores were not significantly different between obese and non-obese groups.[19]

Findings were mixed with regards to gender. Obese girls, compared to obese boys, had significantly more negative perceptions of their physical appearance, self-worth, and how they felt they were accepted by social groups, including their peers. In contrast, no sex differences were found between psychological factors and weight problems, with both sexes reporting the association with low self-esteem and obesity.1 In our study, the number of boys (58%) in the obese group was greater than the number of girls (42%).

Peer relationship problems were greater at age 8-9 years than at younger ages (4-5 years).[20,21] Obese children aged 6 to 13 years were 4 to 8 times more likely to be teased and bullied than normal weight peers. Obesity and weight-related teasing were significant risk factors for the development of psychosocial problems, including social stigmatization or peer rejection, and later eating disorders, and unhealthy weight- control behaviors.[22,23]

The weakness of our study was we did not differentiate nutritional status in non-obese group, as we were not looking for a relationship between total quality of life scores and waist circumference, we also did not make adjustments to other factors that could affect quality of life of early adolescents.

Total PedsQL score in obese and non-obese group was difference significantly in both child and parent-proxy reports. Management of obesity should include health-related quality of life measurements as a parameter of overweight and obesity outcome. We recommend the use of PedsQL questionnaire as a simple, easy-to use, and reliable measurement model for assessment of health-related quality of life. Better understanding quality of life is a key element essential for the treatment of childhood and adolescent obesity.

Conflict of Interest

None declared.

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Original Article

Prevalence of hepatitis and its correlation with serum ferritin and aminotransferase levels among thalassemia major patients in Indonesia

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Abstract

Background Thalassemia major patients who undergo routine transfusion have an increased risk of acquiring transfusion-transmitted infections (TTI), including hepatitis B and C. These diseases have serious implications and may affect the serum ferritin and aminotransferase levels of thalassemia major patients.

Objectives To identify the prevalence of hepatitis B and/or C infections among thalassemia major patients and to evaluate its correlation with serum ferritin and aminotransferase levels.

Methods This was across-sectional study conducted at the Thalassemia Center of Dr. Cipto Mangunkusumo Hospital in Jakarta, Indonesia. The subjects were screened for hepatitis B and C infections, and their serum ferritin and aminotransferase levels were also measured.

Results In total, 621 subjects were included in the study, among which 5 subjects tested positive for hepatitis B surface antigen (HBsAg) (0.8%), 111 subjects tested positive for anti-HCV (17.8%), and 5 subjects tested positive for both HBsAg and anti-HCV (0.8%). The subjects who tested positive for hepatitis B, hepatitis C, or both showed significantly higher values of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum ferritin compared to their negative counterparts. Moreover, serum ferritin showed a positive, moderate correlation with both AST and ALT.

Conclusion This study shows a significant association between hepatitis and serum ferritin as well as aminotransferase levels. Early detection and early management of hepatitis B and C infections is warranted to minimize the occurrence of liver damage in thalassemia major patients. **[Paediatr Indones. 2017;57:176-80; doi: http://dx.doi.org/10.14238/pi57.4.2017.176-80]**.

Keywords: *thalassemia major; hepatitis; serum ferritin; AST; ALT*

Thalassemia is a genetic, blood disorder that requires multiple blood transfusions. Many patients show prolonged survival rates following routine transfusion, but blood transfusions cause iron accumulation in various tissues. One of the organs most prone to iron overload is the liver. Moreover, thalassemia patients are at risk of transfusion-transmitted infections (TTI), including hepatitis B and C. In addition to hepatic iron overload, hepatitis infection further damages liver cells.[1]

The prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection vary among countries. Chronic HBV and HCV infection was estimated to be 240 million and 130-170 million people, respectively, worldwide.[2] The *World Health Organization* (WHO) also noted that HBV is highly endemic to countries in Southeast Asia, including Indonesia.[3] Although the mortality rate due to liver disease in thalassemia patients is low (2.7-4.1%), it should not be considered trivial. Liver cirrhosis

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in thalassemia has been linked to HCV and iron overload.[4,5] Among thalassemia patients worldwide, 0.3-5.7% are hepatitis B surface antigen (HBsAg) positive and 4.4-85.4% are anti-HCV positive.[6]

Indonesia is a developing country, with a majority of the population belonging to the low and middle socioeconomic demographic. Hence, the treatment of hepatitis using antivirals (ribavirin or interferon) is generally considered expensive and to a certain extent, unaffordable. Therefore, blood screening was proposed to prevent TTI. The *Indonesian Ministry of Health* has issued policies regarding blood services and stated that blood should only be collected from voluntary, non-remunerated blood donors, and not for commercial purposes. All blood donors are screened for TTI, including HBV, HCV, human immunodeficiency virus (HIV), and syphilis. Approximately 2.9% of blood products are disposed of due to positive TTI screening results every year.[7]

This study was aimed to describe the prevalence of HBV and HCV infection in thalassemia major patients by antibody detection, as well as to assess for correlations between hepatitis infection and liver transaminase enzyme as well as serum ferritin levels.

Methods

This descriptive, cross-sectional study initially evaluated 1,088 thalassemia major patients who received routine blood transfusions once every 2 – 4 weeks at the Thalassemia Center, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia in 2015. Among these patients, 621 subjects were screened for hepatitis markers and subsequently enrolled in this study. The information collected included subjects' identity, sex, type of thalassemia, age at diagnosis, and age at first transfusion. Nucleic acid testing (NAT) has only been routinely available in four big cities in Indonesia (Jakarta, Bandung, Surabaya, and Denpasar) since 2015, so the data was collected from children who received routine transfusions prior to NAT testing.

Subjects were tested for HBsAg and anti-HCV antibodies, by electrochemiluminescence immunoassays (ECLIA), using the *Cobas e601* analyzer (*Roche Diagnostics*). Serum HBV DNA and HCV RNA tests were not conducted in this study. Serum ferritin level was also analyzed by ECLIA. Alanine and aspartate aminotransferase levels were measured by immunoassay. The ALT and AST levels were considered to be increased if higher than 40 U/L.[8]

The data was analyzed using *SPSS*, SPSS Inc., Chicago, IL and *GraphPad Prism* 6 software. A P value of <0.05 was considered to indicate a significant relationship between variables. Spearman's test was used to evaluate the correlation between two numeric parameters, while the Mann-Whitney and Kruskal-Wallis tests were used to compare two or more categorical groups.

Results

Among the 621 subjects, positive hepatitis markers were found in 121 (19.4%) patients. Positive blood specimens for HBsAg were detected in 5 (0.8%) subjects, anti-HCV in 111 (17.8%) subjects, and for both HBsAg and anti-HCV in 5 (0.8%) subjects.

A total of 121 subjects were studied, comprising of 65 male and 56 female subjects. The age range of subjects was 5-42 years. There were 61 subjects with β-thalassemia and 60 with β-thalassemia/HbE. The medians and ranges of AST, ALT, and serum ferritin levels were 61 (9-194) U/L, 55 (6-218) U/L, and 6,117 (1,395-16,636) U/L, respectively. Increased ALT was found in 81 (66.9%) subjects.

Table 1 shows the characteristics of subjects among the patients positive for HbsAg, anti-HCV, and both. The AST, ALT, and serum ferritin levels were found significantly higher in the hepatitis-positive compared to hepatitis-negative subjects ($P=0.02$, 0.029, and < 0.01, respectively) (**Table 2**). **Figures 1** and **2** show a moderate correlation between aminotransferase and serum ferritin levels among the various groups of subjects positive for hepatitis.

Discussion

We found a higher prevalence of HCV than HBV in thalassemia patients, but past studies have reported varying prevalences of HCV compared to that of HBV in thalassemia patients.9-12 Different assays may yield contrasting results. Purnamawati et al. found that

Table 1. Demographic comparison for subjects with positive HBsAg results, positive anti-HCV results, and both

Characteristics	Positive HBsAg (n=5)	Positive anti-HCV (n=111)	Positive HBsAg + anti-HCV (n=5)
Mean age (SD), years	14.4 (6.6)	24.0 (6.3)	22.0 (7.1)
Gender, n			
Males	4	58	3
Females	1	53	2
β-thalassemia subjects, n	2	55	4
β-thalassemia/HbE subjects, n	3	56	1
Mean age at first diagnosi s of thalassemia, years	7.0	5.3	3.0
Mean age at first transfusion, n			

Table 2. Comparison of AST, ALT, and serum ferritin levels between hepatitis-positive and hepatitis-negative subjects

Variables	Positive HBsAg (n=5)	Positive anti-HCV (n=111)	Positive HBsAg + anti-HCV (n=5)
Median AST (range), U/L	61 (9-194)	48 (14-5190	0.02
Median ALT (range), U/L	55 (6-128)	46 (1-853)	0.029
Median serum ferritin (range), ng/mL	6,177 (1,395-16,636)	3,324 (3-20,831)	<0.0001

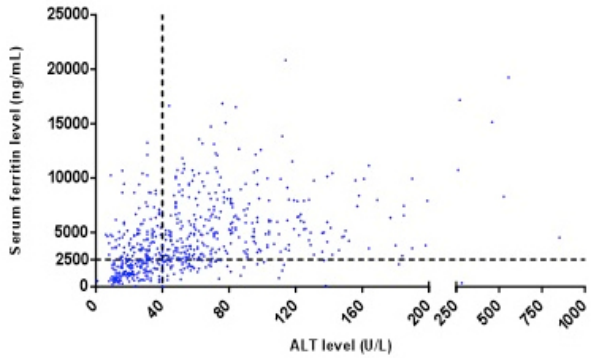

Figure 1. Correlation between ALT and serum ferritin level (r=0.53, P<0.0001)

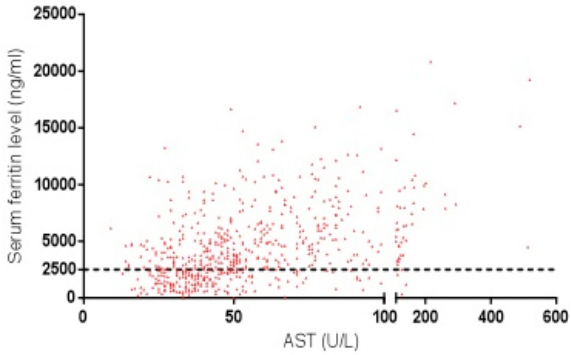

Figure 2. Correlation between AST and serum ferritin level (r=0.46, P<0.0001)

the prevalence of hepatitis C among subjects with thalassemia was 6.7% (6/90). They used the Entebe dipstick anti-HCV test. Among the six subjects with hepatitis C, five had increased ALT levels (>40 U/L), and four had increased transferrin saturation.[9]

A previous study in thalasemmia patients identified positive anti-HCV in 49.5% of subjects and positive HbsAg in 3.2% of subjects, using a commercial ELISA kit (*version-1*, China).[10] Also, Wanachiwanawin et al. found that among thalassemia patients, 2% had positive HBsAg results and 20.2% had positive anti-HCV results. The HBsAg was evaluated using passive hemagglutination assay kits (MyCell, Tokyo, Japan), and anti-HCV by second generation enzyme immunoassay (*EIA II*, Tokyo, Japan).[11] In addition, Vidja et al. found that 2% of thalassemia patients had positive HBsAg results, and 2% had positive anti-HCV results, both of which were detected with the ELISA method.[12] Further assay development is needed to distinguish a reactive result from a weak antigen/antibody concentration.

Electro-chemiluminescence immunoassay was shown to have weak sensitivity and specificity for cut-off indexes (COI) between 1.0 and 4.0, which would, therefore, require confirmatory testing. However, for COIs between 4.0 and 10.0, the confirmatory testing showed the same positive results.[13] Besides the assay kit, the implementation of screening on donated blood is another factor that may account for differences in HBV and HCV infection rates. In the US, hepatitis

C screening has been done since 1992, while in Iran since 1996. It should also be noted that NAT screening began in Indonesia in 2014, but a majority of our patients had received transfusions long before this period. The provision of free NAT testing for patients has only recently been conducted (since 2015).

Hepatitis infection tends to induce iron accumulation in the liver. Therefore, we would expect increased iron profile levels such as serum ferritin, serum iron, and transferrin saturation.[14,15] The hypothesis for this mechanism is that the virus inside liver cells accumulates the iron for its replication, and the immune status of the host response to viral infection may be modified.[16]

Our low numbers of hepatitis B-positive patients may not represent a true low, as our facility can only currently conduct serologic examinations, and it is costly to evaluate viral load and genotype. As such, HBV DNA testing could not be conducted on a routine basis. This limitation also highlights the importance of predicting the true burden of HBV infection. Several studies found the presence of occult hepatitis B (OHB) infections in thalassemia patients. Studies in Egypt and India, for instance, found 32.5-32.8% occult HBV infections in children with thalassemia.[17,18] Moreover, Shaker et al. found OHB in all HCV-infected subjects.[17] Occult hepatitis B is defined as the detection of HBV DNA in patients with serial negative results for HbsAg, with or without hepatitis B antibodies. The diagnosis of OHB requires an assay with high sensitivity and specificity that can detect a low limit of < 10 IU/mL of HBV DNA.[19]

The increase in aminotransferase enzymes may reflect the occurrence of hepatocellular injury. For instance, ALT maintains its highest concentration in the liver, while AST may be found in other organs besides the liver, such as the heart, the kidney, as well as skeletal muscles. Therefore, ALT is more specific as a marker for hepatocellular injury compared to AST. We found a significant correlation between aminotransferase and serum ferritin in patients with hepatitis. An increase in ALT levels could be due to iron overload, alcohol use, or hepatitis virus infection. History of alcohol consumption was not evaluated in these subjects. However, we assumed that a large percentage of subjects were not alcohol abusers since it is restricted by law in Indonesia. Ideally, hepatitis B vaccinations should be provided for all thalassemia patients, to prevent hepatitis B virus infection, which can further contribute to liver failure. As shown in **Table 2**, the median levels of aminotransferase and serum ferritin in hepatitis-positive subjects were higher than those of negative subjects. Wanachiwanawin *et al.* reported that subjects with positive anti-HCV results generally had higher levels of ALT and AST compared to those with negative anti-HCV results.[11] Other factors such as use of iron chelation and frequency of transfusion may also affect the serum ferritin and aminotransferase level.

However, the presence of HCV does not alter the effects of iron overload on liver function. Triantos et al. found that the survival of thalassemia patients was not associated with the presence of hepatocellular carcinoma and other liver diseases. Instead, an association was found between the former and cardiac failure, as well as non-adherence to chelation treatment.[20] Liver MRI is recommended for those patients to more specifically evaluate iron overload. Azarkeivan *et al.* found a moderate correlation between serum ferritin level and relaxation time of liver MRI T2 ($r=-0.535$).[21] Hepatitis B antibody testing was not conducted, nor was hepatitis B vaccination status known in our subjects.

With regards to iron chelation therapy, a study in Indonesia found that a higher dose of deferiprone is required to treat thalassemia major patients with consequent hepatitis B and/or C infections as opposed to hepatitis-negative patients.[22] This, in turn, also highlights the importance of hepatitis detection in thalassemia patients.

In summary, this study demonstrates the current prevalence and clinical significance of HBV and HCV infection among thalassemia patients in Indonesia. The subjects who tested positive for HBV and HCV tended to have higher serum ferritin, AST, and ALT levels than those without infection. Therefore, early and aggressive management should be considered to prevent further liver damage in thalassemia patients with concomitant hepatitis. In the future, now that NAT screening in Indonesia has been routinely practiced since 2015, we hope that the incidence of hepatitis B and C, as well as other infections such as HIV, in thalassemia patients can be drastically decreased.

Conflict of Interest

None declared.

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Original Article

Diagnostic value of newborn foot length to predict gestational age

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Abstract

Background Identification of gestational age, especially within 48 hours of birth, is crucial for newborns, as the earlier preterm status is detected, the earlier the child can receive optimal management. Newborn foot length is an anthropometric measurement which is easy to perform, inexpensive, and potentially efficient for predicting gestational age.

Objective To analyze the diagnostic value of newborn foot length in predicting gestational age.

Methods This diagnostic study was performed between October 2016 and February 2017 in the High Care Unit of Neonates at Dr. Moewardi General Hospital, Surakarta. A total of 152 newborns were consecutively selected and underwent right foot length measurements before 96 hours of age. The correlation between newborn foot length to classify as full term and gestational age was analyzed with Spearman's correlation test because of non-normal data distribution. The cut-off point of newborn foot length was calculated by receiver operating characteristic (ROC) curve and diagnostic values of newborn foot length were analyzed by 2 x 2 table with *SPSS 21.0* software.

Results There were no significant differences between male and female newborns in terms of gestational age, birth weight, choronological age, and newborn foot length (P>0.05). Newborn foot length and gestational age had a significant correlation (r=0.53; P=0.000). The optimal cut-off newborn foot length to predict full term status was 7.1 cm. Newborn foot length below 7.1 cm had sensitivity 75%, specificity 98%, positive predictive value 94.3%, negative predictive value 90.6%, positive likelihood ratio 40.5, negative likelihood ratio 0.25, and post-test probability 94.29%, to predict preterm status in newborns.

Conclusion Newborn foot length can be used to predict gestational age, especially for the purpose of differentiating between preterm and full term newborns. **[Paediatr Indones. 2017;57:181-6; doi: http://dx.doi.org/10.14238/pi57.4.2017.181-6]**.

Keywords: *preterm; foot length; gestational age; newborn*

Gestational age is a major determinant of newborn prognosis. Newborns are categorized as preterm, full term, or post-term neonates. These categories refer to the neonates' gestational age grouping: born at < 37 weeks, at 37 to 41 weeks, or at > 42 weeks, respectively. Early identification of gestational age within 48 hours of birth, especially in differentiating preterm from full term newborns born at home or in remote areas, is a major priority for researchers and public health practitioners in order to reduce global mortality from preterm birth. Mortality can be prevented if preterm newborns are identified earlier and treated with simple interventions such as skin-to-skin contact or kangaroo mother care (KMC), early

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breastfeeding, as well as early infection prevention and treatment.[1-4] Identification of preterm newborns is difficult in community settings, especially in remote areas, where maternal labor is assisted by unskilled health workers or unlicensed midwives. As these birth assistants are unable to predict gestational age, half of all newborns in these settings have unknown gestational age.[5]

Early studies related to newborn anthropometric parameters were performed to identify the correlation between anthropometric cut-off points and birth weight, as well as gestational age. Currently, newborn foot length is studied as an alternative anthropometric measurement to detect low birth weight and preterm status, as no special skill is needed to perform this measurement and preterm newborns are not at risk to hypothermia because of the measurement. Newborn foot length is an easy, quick, and efficient measurement for preterm, critically ill newborns. This measurement technique is not influenced by either subcutaneous fat or biological sex.[6,7,8] This study aimed to analyze the diagnostic value of newborn foot length in predicting gestational age.

Methods

This cross-sectional study was performed in Dr. Moewardi General Hospital, Surakarta from October 2016 to February 2017. Subjects were newborns admitted to the High Care Unit (HCU) of Neonates. Neonates aged 0 to 96 hours whose parents provided written, informed consent were included in this study. Newborns with congenital anomalies of the feet, intrauterine growth restriction (IUGR), extremely low birth weight (birth body weight < 1,000 grams), extremely preterm (gestational age < 28 weeks old), severe asphyxia, or large for gestational age were excluded from this study. Subjects were consecutively collected and the minimum required sample size was calculated by diagnostic test sample formula.

Subjects' right foot lengths were measured twice from heel to big toe, with an iron ruler, calibrated to 0.1 cm precision. The reference standard for gestational age was the *New Ballard Score* (NBS) measurement.[9-12]Measurements were conducted by different resident physicians who were in charge of the HCU. Researchers were blinded to these measurements. The study was approved by the Ethics Committee of the Faculty of Medicine at University of Sebelas Maret, Surakarta, and the Ethics Committee of Dr. Moewardi General Hospital.

The Kolmogorov-Smirnov test was used to assess types of distribution of the investigation parameters. Non-normally distributed data were expressed as median and their corresponding interquartile ranges. Categorical variables were expressed as frequencies and percentages or relative values. Baseline characteristics of subjects (gestational age, birth weight, chronological age, and newborn foot length) were expressed as median values and compared between males and females. Data were analyzed by Chi-square test (categorical data) and Mann-Whitney test (numerical data) using *SPSS 21.0* software for Windows. The correlation coefficient to measure the power of correlation between the independent and dependent variables was analyzed by Spearman's correlation test because of non-normal distribution data. The optimal cut-off newborn foot length to predict gestational age was analyzed by ROC curve. Area under the curve (AUC) was used to assess the power of diagnosis. Diagnostic values such as sensitivity, specificity, positive and negative predictive values, as well as positive and negative likelihood ratios were assessed by 2 x 2 table. Diagnostic ability of newborn foot length was considered to be good for diagnostic parameter > 80%. Reliability of the tool was analyzed by α-Cronbach coefficient (reliable for coefficient > 0.7), while inter-observer variation was analyzed with intraclass correlation coefficient (ICC) (good for ICC value > 0.8) and ANOVA test (good validity for $P > 0.05$).

Results

A total of 152 subjects enrolled in this study. Subjects' baseline characteristics were not significantly different between males and females ($P>0.05$) (**Table 1**). The AUC score based on ROC curve was 0.868 ($P<0.01$) (**Table 2, Figure 1**). The optimal cut-off foot length for full term categorization was 7.1 cm. A comparison of newborn foot length and NBS is shown in **Table 3**. Diagnostic values were calculated based on this table. The identification of preterm newborns with foot length < 7.050 cm had a sensitivity of 75.0%,

which means that 75.0% of preterm newborns (<37 weeks) can be detected by a foot length examination, and a specificity of 98.1% means that there is a 98.1% improbability of full term gestational age (> 37 weeks) in newborns who have a foot length <7.1 cm.

The positive predictive value was 94.3%, which means that for newborn foot length <7.1 cm, the possibility of preterm gestational age was 94.3%. In addition, negative predictive value was 90.6%, which means that for foot length > 7.1 cm, the possibility of full term newborn (> 37 weeks) was 90.6%.

Table 1. Baseline characteristics of subjects

Baseline	Gender		Total	P value
	Males (n=72)	Females (n=80)		
NBS*, n(%)				
< 37 weeks	18 (40.9)	26 (59.1)	44 (28.9)	0.309
≥ 37 weeks	54 (50.0)	54 (50.0)	108 (71.1)	
Chronological age**, n(%)				
0 day	45 (62.5)	46 (57.5)	91 (59.9)	0.541
1 day	25 (34.7)	31 (38.8)	56 (36.8)	
2 days	2 (2.8)	2 (2.5)	2 (2.6)	
3 days	0	1 (1.3)	1 (0.7)	
Median NBW** (range)	36 (20-41)	36 (20-41)	36 (20-41)	0.739
Median foot length** (range), cm	7.5 (5.4-9.0)	7.4 (5.5-8.6)	7.4 (5.4-9.0)	0.282
Median birth weight** (range), grams	3,000 (1,000-4,000)	2,700 (1,150-3,900)	2,875 (1,000-4,000)	-0.084

Note: Categorical variables (gestational age and chronological age) were stated by percentage and analyzed by Chi-square test. Numerical variables (New Ballard Score, foot length, and birth body weight) were stated in median (minimum – maximum) because of non-normal data distribution, and were analyzed by Mann-Whitney test. * categorical variables; ** numerical variables.

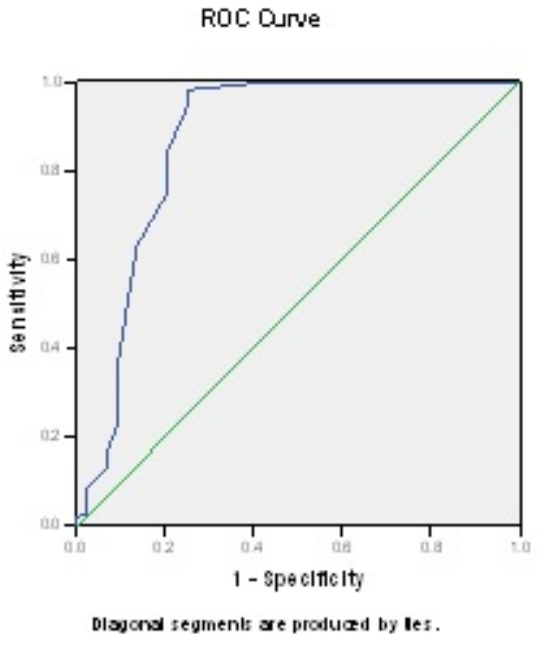

Figure 1. REceiver operating characteristic (ROC) curve.

Table 2. The optimal newborn foot length cut-off point based on New Ballard Score as the reference standard

AUC	Sensitivity	1-Specificity	Cut-off value	P value
0.868	0.981	0.250	7.050	0.000

The positive likelihood ratio (LR+) value was 40.5, indicating that the probability of preterm newborns having a foot length <7.1 cm was 40.5 times greater than foot length > 7.1 cm. In addition, the negative likelihood ratio (LR-) was 0.225, indicating that the probability of preterm newborns having a foot length > 7.1 cm was 0.225 times less than <7.1 cm (**Table 4**).

The correlation analysis between gestational age and newborn foot length is shown in **Table 5**. In male and female newborns, gestational age and foot length had a significant correlation (males: r=0.376; P=0.000; females: r=0.633; P=0.000). Furthermore, regardless of sex, gestational age and foot length had a significant correlation (r=0.533; P=0.000).

Newborn foot length measurements were performed twice by two different physicians. We calculated reliability value of this measurement

with alpha coefficient score. The result was good (r=0.997). ANOVA test also revealed that inter-rater assessment was not significantly different (P=0.903), and inter-class correlation of rater reliability was good (r=0.994).

Table 5. Spearman's rank correlation test results

New Ballard Score	Foot length		
	Males	Females	All subjects
Correlation coefficient	0.376	0.633	0.533

Table 4. Diagnostic study results

Measurement	Diagnostic test					
	Sensitivity	Specificity	PPV	NPV	LR+	LR-
Foot length	0.75	0.98	0.943	0.906	40.5	0.255

Discussion

Preterm newborn birth remains a serious problem and was the highest cause of death in children less than 5 years of age in the last 10 years.[1-4] One of the first steps to assist these newborns is inventing an inexpensive, fast, easy to use, and acceptable screening tool for health workers to identify at-risk babies. Various methods of anthropometry can be performed to diagnose preterm status in newborns, such as the circumferences of the chest, abdomen, head, and calf. However, these measurements are influenced by subcutaneous fat and biological sex. Such measurements also take longer to perform, putting these infants at risk of hypothermia.

Our results are consistent with previous research by Marchant *et al*. in southern Tanzania. They reported that foot length of <8 cm on the first day of birth had a 93% sensitivity (95%CI 82 to 99), 58% specificity (95%CI 53 to 62), 15% positive predictive value, and 99% negative predictive value to detect preterm in newborns. The average foot length on the first day was 7.8 (SD 0.4) cm and on the fifth day was 8.1 cm. The mean difference of foot length between the first day and the fifth was 0.2 cm (SD 0.3).[6]

Ashish *et al.* reported validation of foot length measurements with a ruler as an alternative tool for identifying low birthweight and preterm newborns in a low socioeconomic setting. The cut-off point of 7.2 cm for identifying birth weight <2,000 grams had 75.9% sensitivity and 90.3% specificity, while a 7.8 cm cut-off point to identify premature infants had 76.9% sensitivity, 53.9% specificity, 10.6% positive predictive value, and 90.3% negative predictive value. They also compared the use of a ruler, foot print, and tape measurements, and found that a ruler for measuring foot length had the best AUC (0.683) compared to the other means (foot print 0.680 and tape measure 0.598).[9] Another community-based study by Marchant *et al.* reported on the reliability of the tool to measure newborn foot length. They reported that foot length gauges to classify low birth weight babies were moderately reliable when used by volunteers, with a Kappa score of 0.53 (95%CI 0.4 to 0.66).[7]

Mukherjee *et al.* reported that foot length <7.75 cm had 92.3% sensitivity and 86.3% specificity, for preterm newborn identification. For low birth weight, foot length <7.85 cm had 100% sensitivity and 95.3% specificity. For very low birth weight identification (VLBW), foot length <6.85 cm has 100% sensitivity and 94.9% specificity. A correlation coefficient was calculated by Pearson's correlation test. Foot length and gestational age had a good, positive, linear correlation, with a correlation coefficient of 0.869. Foot length and birth weight also had a good, positive correlation, with coefficient of 0.973 in infants and 0.96 in preterm newborns. They also calculated the sensitivity and specificity of two operational cut-off points: <7 cm had 100% sensitivity and 94% specificity in VLBW identification, while <8 cm had 93.5% sensitivity and 75.3% specificity for preterm identification.[14]

Thi *et al.* reported that foot length <7.4 cm had 85% sensitivity, 86% specificity, 86% positive predictive value, and 84% negative predictive value for diagnosing LBW. Foot length <7.3 cm had 80% sensitivity, 81% specificity, 82% negative predictive value, and 79% positive predictive value for preterm newborns. For LBW along with preterm, newborn foot length <7.3 cm had 86% sensitivity, 83% specificity,

77% positive predictive value, and 90% negative predictive value in making the diagnosis.[16] Srivastata et al reported very strong positive correlation between newborn foot length and gestational age with correlation coefficient 0.99.[15]

Different cut-off points in each study and each country suggests that race or country have a role in determining the cut-off point of the foot length. Factors that influence bone growth during pregnancy include genetics, nutrition, placental supply, and hormones. This study alone has never been done in Indonesia. The results of our study are more specific in diagnosing preterm newborns compared to that of previous studies.

Lee *et al.* examined the clinical assessment validity to determine gestational age of newborns in community setting with a total of 1,066 newborns and had results contrasting to ours. They assessed the accuracy of Ballard score, Capurro technique, Eregie technique, Bhagwat technique, and foot length in predicting gestational age compared to the gold standard of ultrasound at gestational age <20 weeks (first and second trimesters). Newborn foot length <75 mm had 64% sensitivity and 35% specificity for diagnosing preterm status in newborns. They concluded that neonatal anthropometry had poor performance to classify preterm newborns (AUC 0.52-0.8), and that newborn foot length was an inaccurate marker for predicting gestational age, due to the high frequency of intrauterine growth restriction newborns in their setting. They did not exclude IUGR and large for gestational age babies, so these criteria influenced the results.[17]

A limitation of this study was our focus on foot length to predict preterm and full term newborns who were appropriate-for-gestational age (AGA), to the exclusion of IUGR and large-for-gestational age babies. Moreover, our study was done in a hospital-based setting, so the prevalence of preterm was higher than in a community setting. As such, our results may have be biased in terms of foot length compared to the general population, in which the incidence of IUGR babies is present.

In conclusion, newborn foot length can be used to predict gestational age. Longer newborn foot length is indicative of higher gestational age. The optimal cut-off point for diagnosing full term babies was 7.1 cm. Foot length <7.1 cm can be used to diagnose preterm babies. Newborn foot length is a reliable anthropometric measurement to diagnose preterm babies.

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Conflict of interest

None declared.

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Original Article

Effects of *Nigella sativa* oil on Th1/Th2, cytokine balance, and improvement of asthma control in children

Wisnu Barlianto, Maria Rachmawati, Muhammad Irawan, Desy Wulandari

Abstract

Background Asthma is a chronic inflammatory disease of the airways characterized by involvement of a variety of inflammatory cells. Asthma is associated with imbalances between Th1/Th2 cells and their characteristic cytokine profiles. *Nigella sativa* is a plant that possesses immunomodulatory and anti-inflammatory properties.

Objective To investigate the potential anti-asthmatic effect of *Nigella sativa* oil on Th1/Th2 cells, IFN-γ/IL-4 cytokines, and improvement of asthma control.

Methods Children aged 6-15 years with asthma in Dr. Saiful Anwar Hospital, Malang, were enrolled in this study. All patients were treated based on standard treatment guidelines for asthma. *Nigella sativa* oil (NSO) was given per oral as supplementary treatment at a dose of 15-30 mg/kg/day for 8 weeks, in a randomized, single-blind, controlled trial. Peripheral Th1 and Th2 cells were counted by flow cytometry and IFN-γ and IL-4 cytokines were measured by ELISA. Improvement of asthma control was assessed by the asthma control test (ACT) score.

Results Twenty-eight patients completed the study, 14 in the NSO treatment group and 14 in standard treatment group. No significant differences were found in the number of Th1 and Th2 cells, or in the Th1/Th2 ratio between groups after treatment (P=0.074, P=0.481, and P=0.265, respectively). Compared to the control, the NSO group showed a significant elevation of IFN-γ (P=0.046) and reduction of IL-4 (P=0.002). At the end of study, ACT score was not significantly different between groups (P=0.413).

Conclusion Supplementation with *Nigella sativa* oil improves IFN-γ/IL-4 balance and asthma control in children with asthma. **[Paediatr Indones. 2017;57:223-8 ; doi: http://dx.doi.org/10.14238/pi57.5.2017.223-8]**.

Keywords: *Nigella sativa; IFN-γ/IL-4; Th1/Th2; ACT score; asthma*

Asthma is a chronic inflammatory disease of the airways characterized by wheezing, difficulty breathing, or repetitive paroxysmal cough. Airway inflammation and hyperresponsiveness are central pathogenic features of asthma.[1] This process has a complex pathogenesis involving both genetic and environmental factors. One of the mechanisms underlying airway inflammation is an imbalance in T helper immune cells.[2] Experimental and clinical data suggest that the balance between the Th1 and Th2 cellular responses are central to the pathogenesis of allergic airway inflammation.[3-5] It is widely accepted that Th2 cytokines such as interleukin (IL)-4, IL-5, IL-9, and IL-13 play critical roles in orchestrating and amplifying allergic inflammation, while the Th1 cytokines, such as IFN-γ and IL-12, are thought to prevent this process.[6] In asthma, T helper type 2 (Th2) cells are functionally upregulated, while Th1 cells are inhibited, which enables Th2 cytokines to promote

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inflammation. Interleukin-4 (IL-4), secreted by Th2 cells, induces airway inflammation by activating eosinophils and promoting IgE secretion.[7] Therefore, one effective treatment for asthma is to improve Th1 immune responses and simultaneously inhibit Th2 immune responses to restore Th1/Th2 balance.[8]

One important goal of asthma treatment is to control the disease. Poor compliance with conventional asthma medications remains a major problem in achieving asthma control. Other problems are that some patients do not respond to intense asthma medications or they experience many undesired side effects due to long-term use of the medication.[8] The *Global Asthma Physician and Patient* (GAPP) Survey reported that 39% of asthma patients exchanged or stopped their asthma medication because of adverse events.[9] For this reason, introduction of novel treatment strategies is a key step for better asthma control.

Black seed or *Nigella sativ*a reportedly has anti-inflammatory and anti-allergy effects.[10] For thousands of years, *Nigella sativa* has been traditionally used as a spice, food additive, preservative, and herbal remedy for various diseases.[11] In several clinical studies, *Nigella sativa* showed positive effects on clinical and biochemical markers of asthma inflammation.[12,13] In mouse models of asthma, *Nigella sativa* oil (NSO) reduced airway hyperresponsiveness, total leukocytes, macrophages, eosinophils, and serum levels of total immunoglobulin E (IgE).[14] The aim of this study was to investigate the potential anti-asthmatic effect of *Nigella sativa* on IFN-γ/IL-4 cytokines, Th1/Th2 cells, and improvement of asthma control in children with asthma.

Methods

Twenty-eight children aged 6-15 years diagnosed with asthma in the Department of Allergy and Immunology, Dr. Saiful Anwar Hospital, Malang, were recruited between February and December 2013. Asthma diagnosis and assessment of severity were performed according to the *Global Initiative for Asthma* (GINA) guidelines.[1] Subjects were randomly divided into either the treatment or the control group. This study was a single-blind, controlled trial, which means that only the subjects were blinded to the identity of the group. This study was approved by the Ethics Committee of the Brawijaya University Medical School. Written informed consent was obtained from parents/guardians of all subjects.

In this study, we used softgel capsules containing 500 mg *Nigella sativa* oil (NSO), with brand name Minyak Habbatussauda MADINAH and licensed as an herbal medicinal product in Indonesia (POM TR.123 329 761). All patients were on routine asthma medications according to GINA guidelines for standard asthma management.[1] These included inhalation of β2 agonist for intermittent asthma and β2 agonist + corticosteroid inhalation for persistent asthma. In the treatment group, NSO was given as adjunctive therapy at a dose of 15-30 mg/kg/day for 8 weeks.

Peripheral blood mononuclear cells (PBMCs) of heparinized peripheral blood from the study subjects were isolated by *Ficoll* density gradient centrifugation. The cells were cultured in RPMI 1640 medium (*Invitrogen*) supplemented with 100 U/mL penicillin, 100 U/mL streptomycin, 2 mM glutamine, and 10% fetal bovine serum (FBS) (*Gibico*, USA). The PBMCs were harvested, washed, and stained with fluorescein isothiocyanate (FITC)-conjugated anti-human CD4 for 30 minutes (*BioLegend*, San Diego, CA). After surface staining, the cells were stained with phycoerythrin (PE)-conjugated anti-human IFN-γ (*BioLegend*, San Diego, CA) for Th1 detection, and PE-conjugated anti-human IL-4 (*BioLegend*, San Diego, CA) for Th2 detection, at 4°C for 30 minutes. Data were acquired on a FACS Calibur and analyzed using flow cytometry software.

Serum specimens were obtained from peripheral venous blood that was centrifuged at 3000 rpm for 5 min. The measurement of serum IFN-γ and IL-4 level was done by human ELISA kit (*Novateinbio Human IFN-γ ELISA Kit* and *Novateinbio Human IL-4 ELISA Kit*).

For assessment of asthma control, we used the *Asthma Control Test* (ACT) questionnaire. The ACT was one of the best-validated instruments to measure asthma control, consisted of five questions on a total scale of 5–25, with each question scaled from one to five. Full control was defined as a total ACT score of 25.[1]

Statistical analysis was performed by *SPSS* for *Windows* version 16.0 (*SPSS Inc*., USA). Data were

expressed as mean (SD). Statistical analyses were performed using paired and unpaired T-test to evaluate the differences between groups. The correlation coefficient was generated by Pearson's correlation. Statistical significance was defined to be P values <0.05.

Results

The clinical characteristics of participants are summarized in **Table 1**. There were 14 children in the NSO treatment group and 14 children in the standard treatment group. During our study, all patients completed the study and there were no treatment side effects observed. The subjects' mean ages were similar in both groups. The subjects were predominantly female and most had good nutritional status. More than 50% percent of subjects had a family history of atopic disease, such as rhinitis, asthma, eczema, urticaria, and conjunctivitis. The most common clinical manifestation was cough and dyspnea. The assessment of asthma severity revealed 50% with intermittent asthma and 50% with mild persistent asthma, in each group.

Table 1. Baseline characteristics of subjects

Characteristics	NSO treatment group (n= 14)	Standard treatment group (n= 14)
Mean age (SD), years	8.79 (2.940)	8.71 (3.771)
Gender, n		
Male	5	6
Female	9	8
Nutritrional status, n		
Good	12	13
Underweight	2	1
Family history of atopic disease, n		
Yes	10	9
No	4	5
Clinical manifestation, n		
Cough	2	2
Dyspnea	4	5
Cough + dyspnea	8	7
Asthma classification, n		
Intermittent	7	7
Mildly persisten	7	7

No significant differences between groups were found in the numbers of Th1 cells after 8 weeks of treatment [19.335 (SD 7.328) *vs.* 20.577 (SD 13.273), P=0.074]. But the number of Th1 cells tended to increase in both groups post-treatment. There was also no significant differences between groups in the numbers of Th2 cells after 8 weeks of treatment [21.875 (SD 12.871) *vs.* 18.478 (SD 11.729), P=0.481], (**Figure 1**). Th1/Th2 ratio showed no significant difference in the standard and NSO treatment groups [1.459 (SD 1.292) *vs.* 1.994 (SD 2.135), P=0.265], but Th1/Th2 ratio was higher in the NSO treatment group.

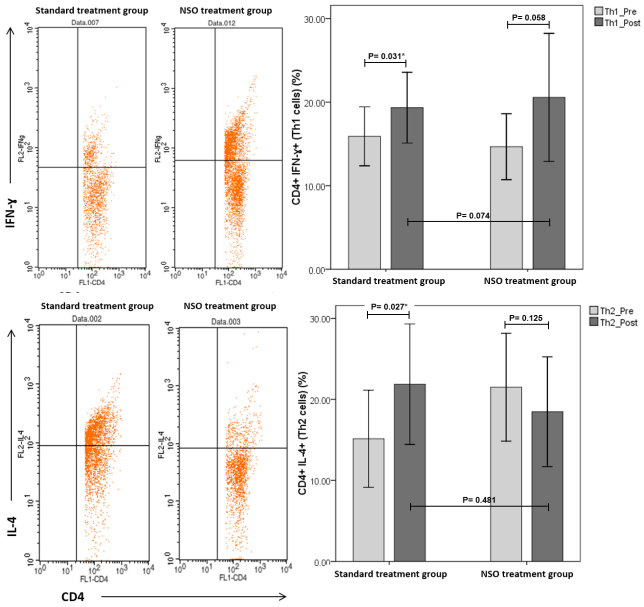

Figure 1. The number of Th1 and Th2 cells in the standard and NSO treatment groups

At the end of study, serum IFN-γ levels were significantly increased in the NSO group, while IL-4 was markedly decreased in the NSO group, compared to the standard treatment group (P=0.046 and P=0.002, respectively). At baseline, there were no significant differences in serum IFN-γ and IL-4 levels between groups before treatment (P=0.575 and P=0.470, respectively). In addition, there was no significant difference in IFN-γ/IL-4 ratio between groups both before and after treatment (P=0.275 and P=0.130, respectively) (**Table 2**).

Table 2. Serum levels of IFN-γ and IL-4 cytokines

Variables	Pre-treatment			Post-treatment		
	Standard treatment group	NSO treatment group	P value	Standard treatment group	NSO treatment group	P value
Mean IFN-γ (SD), pg/mL	10.083 (3.190)	12.495 (4.367)	0.575	9.786 (3.273)	20.035 (6.416)	0.046
Mean IL-4 (SD), pg/mL	1.303 (0.519)	1.413 (0.331)	0.470	1.434 (0.512)	1.107 (0.207)	0.002
Mean IFN-γ/IL-4 ratio (SD)	8.959 (4.738)	10.421 (4.663)	0.275	9.476 (3.834)	20.516 (5.700)	0.130

After 8 weeks of treatment, the mean ACT score was not significantly different in the standard and NSO treatment groups (P=0.692). But, there were significant increases in ACT scores between pre- and post-treatment in the standard treatment group [17.57 (SD 1.222) *vs.* 19.36 (SD 1.151), respectively, P<0.0001)] and the NSO treatment group [16.57 (SD 2.533) *vs.* 20.29 (SD 1.816), respectively, P<0.0001)] (**Figure 2**).

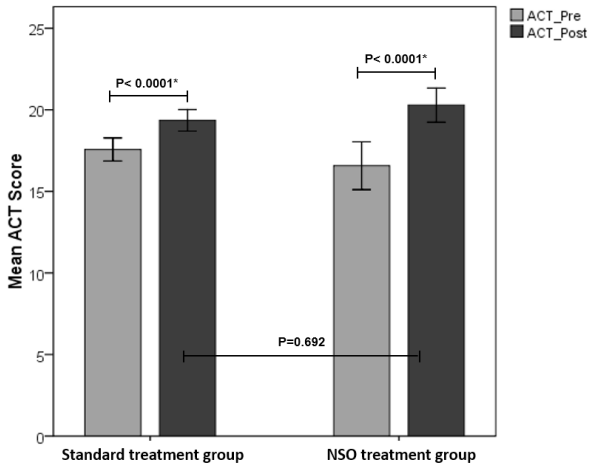

Figure 2. The mean ACT scores in the standard and NSO treatment groups, pre- and post-treatment

Although the results of this study showed an increase of IFN-γ, decrease of IL-4, and improvement of ACT score in the NSO group, we found no significant correlations between IFN-γ, IL-4, IFN-γ/IL-4 ratio, and ACT score. The number of Th1 cells, Th2 cells, and the Th1/Th2 ratio also showed no correlation with ACT score (**Table 3**).

Table 3. Correlation between biochemical markers and ACT score in the NSO group

Variables	r	P value
IFN-γ	0.081	0.681
IL-4	0.018	0.927
IFN-γ/IL-4 ratio	0.049	0.803
Th1	0.070	0.722
Th2	0.152	0.278

Discussion

Nigella sativa (NS) or commonly known as black seed is a one of medicinal plants that belongs to Ranunculaceae Family. The seeds and oil of NS have been widely used for the treatment of various diseases. NS has been extensively studied for its biological activities and therapeutic potential and shown to possess wide spectrum of activities such as anticancer and immunomodulatory, analgesic and anti-inflammatory, anti-allergy, antimicrobial, anthelmintics, spasmolytic, bronchodilator, gastroprotective, hepatoprotective and antioxidant properties.[15] A randomized, double-blind, placebo-controlled trial conducted by Koshak *et al.* showed that NSO improved the mean ACT score in children with asthma.[13] Our previous study reported that the administration of *Nigella sativa* oil along with immunotherapy and probiotic therapy to asthmatic children, significantly increased their ACT scores.[12] Another study described that children with asthma who were given NSO showed a significant reduction in pulmonary index (PI) and improvement of peak expiratory flow rate (PEFR).[11] Boskabady *et al.* also reported that prophylactic therapy with an aqueous extract of NS could improve the severity of asthma symptoms.[16] However, in our study, both groups had significantly improved ACT scores pre-

and post-treatment. These improvements were not significantly different between the two groups.

Several mechanisms of action have been suggested for *Nigella sativa*, which may explain its beneficial effects in asthma. One such mechanism is the regulation of Th1/Th2 cellular balance. In allergy conditions, such as in asthma, regulation of Th1 and Th2 cells is a key process contributing to asthma pathogenesis.[3] Th2 cells promote the macrophage activity and regulate the proinflammatory response, whereas Th1 cells inhibit the activity of Th2. The Th1 cells produce IL-2 and IFN-γ, whereas Th2 cells produce IL-4 and IL-10.[6] Interleukin-4 (IL-4), secreted by Th2 cells, induces airway inflammation by activating eosinophils and promoting IgE secretion.[7] Majdalawieh *et al.* described that an aqueous extract of *Nigella sativa* reduces the secretion of Th2 cytokines by splenocytes.[17] An in vivo study also reported that an aqueous extract of *Nigella sativa* increased IFN-γ and decreased IL-4 cytokines, but histopathological findings in lung parenchyma of ovalbumin-sensitized guinea pigs were not improved.[14] Clinical study conducted by Salem *et al.* reported that there was significant increase in the serum IFN-γ and improvement in the ACT score after 12 weeks of *Nigella sativa* supplementation in asthmatic patients.[18]

This study showed that NSO treatment increase of IFN-γ and decrease of IL-4 serum level. It indicates that there was stimulatory effect on Th1 cells and inhibitory effect on Th2 cells. The underlying mechanism of Th1/Th2 cytokine modulation by *Nigella sativa* may be attributed to the inhibition of the signal transducer and activator of transcription-6 (STAT6) signaling pathway.[19] STAT proteins are a group of transcription factors that transmit signals from cytoplasmic milieu of cells to nucleus. The activation of STAT6 is pivotal in naive CD4+T (Th0) cell differentiation to Th2 pathways. At the same time, STAT-6 induction inhibits Th1 differentiation, both by increasing IL-4 production and by inhibiting the master Th1 transcription factor.[20,21]

However, we found no significant differences in the numbers of Th1 and Th2 cells in both groups after 8 weeks of treatment (**Figure 1**). But the number of Th1 cells and Th1/Th2 ratio tended to be increased in the NSO treatment group. It was reported that *Nigella sativa* can potentially inhibit Th2 immune responses, but there was no clear evidence that *Nigella sativa* decreased Th2 cell population. Several clinical studies reported a significant increase in the percentage of CD4+ and CD8+ T cells producing IL-4 in asthmatic children.[22,23] This finding suggests that IL-4 is not only produced by CD4+ Th2 cells, but also by CD8+ T cells. While it is well established that CD4+ T lymphocytes play a crucial role in the initiation, progression, and persistence of asthma, the role of CD8+ T cells is less understood.[23] CD8+ T cells form functionally similar subsets which exhibit similar cytokine profiles as Th1 and Th2 cells, known as Tc1 and Tc2. Evidence from animal studies suggest that CD8+ T cells are capable of regulating IgE production through the induction of IL-12 and IL-18 production in dendritic cells, and that CD8+ T cells may act to moderate Th2 polarization within the localized lymph nodes during allergic sensitization.[24]

Despite the many published studies evaluating immune functions of *Nigella sativa* products, this plant is not yet in clinical use for treatment of asthma. With regards to immunomodulatory effects of *Nigella sativa* and its constituents, this plant has a potential therapeutic effect on asthma. However, more studies are required for clinical application of *Nigella sativa*.

In conclusion, the results indicate that there is no different in ACT scores and Th1/Th2 balance between NSO and standard treatment group, but there is decrease of IL-4 and increase of IFN-γ serum level in asthmatic patients with NSO supplementation.

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Conflict of Interest

None declared.

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Original Article

Gross motor dysfunction as a risk factor for aspiration pneumonia in children with cerebral palsy

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Abstract

Background Respiratory problems, such as aspiration pneumonia, are major causes of morbidity and mortality in children with cerebral palsy (CP) and greatly affect the quality of life of these children. Nevertheless, there is limited data on the incidence and risk factors of aspiration pneumonia in children with CP in Indonesia.

Objective To determine the incidence and risk factors of aspiration pneumonia in children with cerebral palsy.

Methods In children with CP aged 1-18 years, incidence of pneumonia was studied prospectively for 6 months and the prevalence of the risk factors was studied cross-sectionally. At baseline, we evaluated subjects' by history-taking, physical examination, risk factors, and chest X-ray to assess the incidence of silent aspiration. Subjects were followed-up for six months to determine the incidence of overt or silent aspiration pneumonia.

Results Eight out of 36 subjects had one or more episodes of aspiration, consisting of silent aspiration (2/36) and clinically diagnosed aspiration pneumonia (7/36). Subjects with more severe gross motor dysfunction experienced more episodes aspiration pneumonia, although it was not statistically significant (P=0.06), while dysphagia (P=0.2) and nutritional status (P=0.11) were not associated with pneumonia or silent aspiration.

Conclusion Twenty-five percent of children with CP experience aspiration pneumonia during the 6-month study period, with gross motor dysfunction as a possible risk factor. **[Paediatr Indones. 2017;57:229-33 ; doi: http://dx.doi.org/10.14238/pi57.5.2017.229-33]**.

Keywords: *aspiration; pneumonia; cerebral palsy*

Cerebral palsy (CP) is the most common disability in children. In the United States, the incidence is 2-2.5 children per 1000 live births. During January-August 2012, 20 new patients were diagnosed with cerebral palsy in Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia. Children with CP have many complications related to the disease, such as respiratory problems, oromotor dysfunction, gastrointestinal problems, seizures, and mental disabilities.[1]

Respiratory problems, such as aspiration pneumonia, hypoventilation, sleep apnea, and recurrent respiratory tract infection, are often neglected in children with CP. These problems may affect their quality of life and become major morbidities.[2] Approximately 77% of deaths in children with neurological impairment were caused by pneumonia. Incidence of aspiration pneumonia may be as high as 41.5%.[3] Nevertheless, little is known on the incidence and risk factors for aspiration

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pneumonia in children with CP in Indonesia, where awareness of these respiratory problems is low.

Methods

In this study, incidence of pneumonia was studied prospectively and the prevalence of the risk factors was studied cross-sectionally in children with CP. The subjects ranged from 1 to 18 years of age. Subjects with risk factors for aspiration, such as Down syndrome, facial anomalies, tracheostomy, tracheoesophageal fistules, laryngomalacia, and severe intellectual disabilities, were excluded. This study was approved by the University of Indonesia Ethics Committee.

Forty-four subjects were consecutively recruited at the Pediatric Neurology Clinic and Ward of Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia in March 2015. At baseline, we evaluated children by history-taking, physical examination, noting risk factors using a dysphagia questionnaire, and chest X-ray during the first visit to assess the incidence of silent aspiration. Subjects were followed-up for six months from April 1 to September 30, 2015 to determine the incidence of aspiration pneumonia. On each visit, the patient was evaluated for signs and symptoms of aspiration. Diagnosis was established by signs and symptoms of aspiration pneumonia and confirmed by chest X-ray examination. Patients with aspiration pneumonia were treated accordingly.

We used the *Statistical Package for Social Science (SPSS) version 20* in all analyses. Analysis of the risk factors contributing to aspiration pneumonia was done by Fisher's exact and Mann-Whitney tests. Multivariate analysis was done by logistic regression test.

Results

Forty-four patients met the study criteria, but four were excluded. Therefore, a total of 40 subjects were followed-up.

The majority of subjects were male, with a male to female ratio of 1.35:1. The Subjects' median age was 3.5 (range 1.3 to 14.2) years. The most common types of CP were spastic (35/40) and quadriplegic (33/40). Most of the subjects had *Gross Motor Function Classification System* (GMFCS) scale of V (34/40). An almost equal proportion of subjects used a nasogastric tube (NGT) and more than half of them had good nutritional status. The majority of subjects had undergone medical rehabilitation (28/40). Subjects' characteristics are shown on **Table 1**.

Table 1. Subjects' characteristics

Characteristics	N=40
Gender, n (%)	
Male	23 (58)
Female	17 (42)
CP type, n (%)	
Diplegia	6 (15)
Quadriplegia	33 (82)
Monoplegia	1 (3)
Spastic	35 (60)
Hypotonic	5 (40)
Dysphagia, n (%)	
Yes	39 (98)
No	1 (2)
GMFCS level, n (%)	
I	0 (0)
II	1 (2)
III	0 (0)
IV	5 (13)
V	34 (85)
Nutritional status, n (%)	
Severe malnutrition	4 (10)
Mild to moderate malnutrition	13 (33)
Good	21 (53)
Overweight	1 (2)
Obese	1 (2)
Using NGT, n (%)	
Yes	19 (48)
No	21 (52)
Had undergone medical rehabilitation, n (%)	
Yes	28 (70)
No	12 (30)

One subject died in the third month of follow-up (probably due to meningitis) and another subject died in the fifth month of follow-up (probably due to sepsis). Thus, they were considered to have dropped out from this study. Two other subjects were lost to follow-up because the family moved to another city. Hence, 36 subjects completed the follow-up for six months. Two subjects had silent aspiration. Aspiration pneumonia was clinically diagnosed in 7 episodes of 36 subjects. These episodes were not evident in the radiological examination, but 6 out of 7 chest X-rays were done within less than 6 hours of the predicted aspiration event.

Risk factors for aspiration analyzed in this study were GMFCS scale, dysphagia, and nutritional status. None of the risk factors was found to be significantly associated with aspiration (**Table 2**).

Table 2. Risk factors of aspiration in children with CP

Risk factors	Aspiration (n=8)	No aspiration (n=28)	P value
GMFCS level			
II	1	0	0.06
IV	2	3	
V	5	25	
Oromotor dysfunction			
Yes	7	28	0.22
No	1	0	
Nutritional statu			
Wasting	1	13	0.12
Good	7	15	
Using NGT			
No	3	13	0.70
Yes	5	15	
Physiotherapy			
No	4	5	0.09
Yes	4	23	

Discussion

Almost all subjects completed the follow-up and the drop out or loss to follow-up rate was minimal. Subjects were followed up for six months to detect at least one episode of pneumonia in children with CP. This study is the first prospective cohort study to evaluate the incidence and risk factors of aspiration pneumonia in children with CP in Indonesia. A previous study had shown that a retrospective study was not a reliable design for detecting pneumonia, thus, we chose a prospective cohort as the best design.[4]

A limitation of this study was our small sample size and that dysphagia was evaluated using a modified questionnaire, which only gave the general picture of dysphagia. There are various validated surveys for patients with developmental delay and intellectual disability, such as the *Dysphagia Disorder Survey*.[5] Only two patients underwent flexible endoscopic evaluation of swallowing to evaluate for dysphagia. A second limitation of this study was that chest X-ray performed only once, during the first visit. As such, we could not evaluate for silent aspiration occurring during the second to sixth months. Furthermore, gastroesophageal reflux as another risk factor for aspiration was not evaluated in this study.

Most subjects in our study were male (23/40). Male biological sex is a risk factor for CP, as explained by a previous study.[6] The median age of subjects was 3.5 years. Another study in Indonesia found that the mean age at CP diagnosis was 28.8 months.[7] The majority of subjects in this study had GMFCS level V (30/36) and spastic quadriplegic type of CP. This study was conducted in the top tertiary hospital which explained why most study subjects had the severe form of CP and gross motor function level. Almost all subjects had dysphagia (35/36). Silent aspiration was found in 2 out of 36 subjects. Subjects did not undergo a videofluoroscopic swallowing study to detect aspiration; therefore, it is possible that more subjects experienced silent aspiration. There were 7 episodes of clinically diagnosed aspiration pneumonia in 36 subjects. Other studies reported that the incidence of aspiration pneumonia in children with CP was estimated to be 27-41.5%, and could even be as high as 90%.[3,8-9] One subject experienced two episodes of aspiration pneumonia in the six months of follow-up.

Aspiration is often undetected in children with CP. Three out of six subjects had witnessed episodes of aspiration, but almost all subjects had no pathognomonic chest X-ray to confirm aspiration pneumonia, probably due to the chest X-rays being performed within less than six hours after the aspiration episodes. Thus, the diagnosis of aspiration pneumonia was made based on clinical findings.

Risk of aspiration may decrease after nutritional, medical, and behaviorial modification. Patients with dysphagia were given liquid or modified solid food. A nasogastric tube is needed in patients with known risk factors of aspiration, after nutritional and behaviorial modification.[10] Almost half of the subjects in this study had apparently undergone nutritional modification, which could decrease the incidence of aspiration in this study. The majority of subjects had also undergone physical and oromotor rehabilition. These steps could help airway clearance and, as a result, prevent aspiration.[2]

The GMFCS shows the level of motor developmental delay. We found that subjects with more severe gross motor dysfunction experienced more episodes of aspiration pneumonia, although it

was not statistically significant (P=0.06). Gross motor function may worsen or improve over time. Children with CP who underwent physical rehabilitation for 6, 12, and 18 months showed improved gross motor function measures.[11,12] The follow-up of risk factors for aspiration pneumonia should be done continuously, as the level of gross motor function is dynamic, especially after physical rehabilitation. Unfortunately, we did not monitor whether the risk factors evolved during the six months of study.

Oromotor dysfunction is common in children with CP.[13] Recurrent aspiration due to oromotor dysfunction in children with CP may cause chronic cough, breathing problems during sleep, airway colonization by pathogens, and progressive lung parenchymal destruction, which leads to death.[14] Almost all subjects in our study had oromotor dysfunction, which may explain why we found no significant association between oromotor dysfunction and aspiration in our study.

Malnutrition in children with CP is caused by swallowing problems, gastroesophageal reflux, and increased energy expenditure.[15,16] These conditions result in muscle catabolism, including respiratory muscles, which decreases lung function and makes children with CP prone to pneumonia.[2,17] Previous studies in Myanmar and Indonesia found high prevalences of malnutrition in children with CP.[18,19] In contrast, we found that more than half of the subjects had good nutritional status. This finding may be due to many of our subjects having received nutritional modification training. We also did not find a significant association between aspiration pneumonia and nutritional status.

In conclusion, 25% of subjects with CP in our 6-month study have episodes of aspiration, either silent aspiration or aspiration pneumonia. Gross motor dysfunction may be associated with aspiration pneumonia in children with CP.

Conflict of Interest

None declared.

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Original Article

Serum 25-hydroxyvitamin-D level and atopic dermatitis severity in children

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Abstract

Background Vitamin D plays an important role in the immune system. It inhibits B-lymphocyte proliferation and modulates the humoral response to suppress IgE production. Studies on the relationship between serum 25-hydroxyvitamin-D level and the severity of atopic dermatitis in several countries have had varying results.

Objective To assess for a possible correlation between serum 25-hydroxyvitamin-D level and atopic dermatitis severity in children.

Methods A cross-sectional study was conducted in 26 children with atopic dermatitis from September to December 2015. We evaluated the severity of disease using the *Scoring of Atopic Dermatitis* (SCORAD) index and measured serum 25-hydroxyvitamin-D levels. Spearman's test was used to analyze for a correlation between serum 25-hydroxyvitamin-D level and the atopic dermatitis score in children with atopic dermatitis.

Results Mean SCORAD index was 32.0 (SD 14.99) , with a range of 10.9 to 71.4. Mean serum 25-hydroxyvitamin-D level was 41.1 (SD 24.81) ng/mL, with a range of 10-137 ng/mL. There was a moderate correlation between serum 25-hydroxyvitamin-D level and the SCORAD index (r=-0.591), with higher SCORAD index associated with lower serum 25-hydroxyvitamin-D level (P=0.01).

Conclusion There is a moderate correlation between serum 25-hydroxyvitamin-D level and the SCORAD index in children with atopic dermatitis. **[Paediatr Indones. 2017;57:234-8; doi: http://dx.doi.org/10.14238/pi57.5.2017.234-8]**.

Keywords: *atopic dermatitis; vitamin D; children*

Atopic dermatitis (AD) is the most prevalent skin disease in infants and children and is characterized by an inflammatory reaction on the skin in response to hereditary and environmental factors.[1] The incidence was estimated to be 15-30% in children, with 85% affected before the age of 5 years and 2-10% affected as adults. In genetically at-risk babies, the onset in 48-65% of cases was in the first 6 months of life, 57% before 4 months of life, and 75-80% within the 1st year, with male prevalence higher than female.[2] Atopic dermatitis has increased by 2- to 3-fold during the past 3 decades in industrialized countries.

The pathogenesis of AD is not completely understood, however, the disorder appears to result from a complex interaction between defects in skin

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barrier function, immune abnormalities, as well as environmental and infectious agents.[3] Hanifin and Rajka's criteria is the gold standard for diagnosing AD, defined as fulfilling 3 out of 4 major criteria or 3 out of 23 minor criteria.[4] The severity of disease can be evaluated by the SCORAD index. The SCORAD index consists of interpreting the severity of the disorder by 3 criteria: extent (the percentage of the skin surface affected according to the rule of nines), intensity (composed of erythema, edema/papules, effect of scratching, oozing/crust formation, lichenification, and dryness), and subjective symptoms (composed of pruritis and sleeplessness.[5]

Vitamin D is a hormone with multiple physiologic actions, the metabolites of which are stored in tissue and circulate in plasma.[6] Vitamin D has been shown to inhibit B-lymphocyte function and modulate the humoral immune response, resulting in diminished secretion of IgE.[7] Calcidiol or 25-hydroxyvitamin-D [25(OH)D] is the most common vitamin D metabolite in human serum, with a half-life of 3 weeks in serum so it is considered to be an acceptable proxy for vitamin D level in the body.[8]

Several studies reported a correlation between vitamin D and atopic dermatitis. A small study of 37 children with atopic dermatitis found a significant correlation between serum 25(OH)D level and severity score for atopic dermatitis.[9] A Chinese study in 2013 reported a significant correlation between atopic dermatitis and low serum level of 25(OH) D in children. Also, the total IgE was found to be higher in patients with low levels of 25(OH)D.[10,11] But a study in Milwaukee in 2013 reported that serum 25 hydroxyvitamin D did not correlate with atopic dermatitis severity, with lower serum 25(OH) D concentration in mild AD cases compared to moderate and severe AD cases.[12]

The differing study results spurred us on to assess for a correlation between serum 25-hydroxyvitamin-D level and SCORAD index in children with atopic dermatitis in Medan, North Sumatera, especially in the Helvetia health clinic (Puskesmas), a tropical environment with a mid-low socioeconomic population.

Methods

This cross-sectional study was conducted on 26 children aged ≤ 5 years with atopic dermatitis who visited the Helvetia health clinic (*Puskesmas*) in Medan, North Sumatera, from September to December 2015. Diagnosis of AD was estabished by Hanifin and Rajka's criteria.[4] We evaluated the severity of disease using the SCORAD index. Serum 25-hydroxyvitamin-D level was obtained from laboratory by using *@Alegria* machine. The Sample of blood is taken from vena mediana cubiti about 1.5 mL.

The SCORAD index was developed by the *European Task Force on Atopic Dermatitis* (ETFAD).[13] To measure the extent of AD, the rule of nines is applied on a front/back drawing of the patient's inflammatory lesions, and graded on a scale of 0-100. The intensity criterion of the SCORAD index consists of six items: erythema, edema/papules, excoriation, lichenification, oozing/crusting, and dryness. Each item can be graded on scale of 0-3. (0=no, 1=mild, 2=moderate, 3=severe). The subjective criterion includes daily pruritus and sleeplessness, was graded on a visual analogue scale of 1-10, with a maximum subjective score of 20. All items should be filled out on the standardized SCORAD evaluation form. The SCORAD index formula is A/5 + 7B/2 + C, with A defined as extent (0-100), B defined as intensity (0-18), and C defined as subjective symptoms (0-20). Total SCORAD index < 25 was defined as mild, 25-50 was defined as moderate, and >50 was defined as severe.[5]

Data distribution was evaluated using Shapiro-Wilk's test. Spearman's test was used to analyze for a correlation between serum 25-hydroxyvitamin-D level and SCORAD index in children with atopic dermatitis. Results with P values < 0.05 were considered to be statistically significant, with 95%confidence intervals (CI).

Results

The characteristics of subjects are shown in **Table 1**. The characteristics consisted of sex, age, birth order in family, body weight, body hight, nutrition, family history of atopy and the SCORAD index. We found

that atopic dermatitis in male more prevalence than female, and children with atopic dermatitis in the < 1 year old group was bigger than the 1-5 year old group. Regarding birth order, the first and the second children were more likely to have atopic dermatitis. Most subject had normoweigth. We also found children with AD more prevalence in atopic of one of parent than both. The most prevalence SCORAD index of the children was moderate.

Table 2 shows the mean (SD) SCORAD index and serum 25-hydroxyvitamin-D levels, as well as range of values in children with atopic dermatitis. Mean of the SCORAD Index was 32 (SD 14.99), with the lowest score of 10.9 and the higher score was 71.4. Mean of the serum 25-hydroxyvitamin-D level was 41.06 (SD 24.81) ng/mL, with the lowest level of 10 ng/mL and the higher level of 137 ng/mL.

There was a moderate, negative correlation between serum 25-hydroxyvitamin-D level and the SCORAD index (P=0.01), with higher SCORAD index associated with lower serum 25-hydroxyvitamin-D level (r = - 0.591) (**Figure 1**).

Table 1. Characteristics of subjects

Characteristics	N=26
Gender, n	
Male	16
Female	10
Age, n	
< 1 year	11
1-5 years	15
Birth order in the family, n	
1st	10
2nd	10
3rd	5
4th	1
Mean body weight (SD), kg	10.4 (4.85)
Mean body height (SD), cm	78.0 (19.03)
Nutrition, n	
Overweight	2
Normoweight	22
Moderate malnutrition	1
Severe malnutrition	1
Family history of atopy, n	
None	9
Father only	1
Mother only	8
Mother and father	3
Siblings	11
SCORAD index, n	
Mild	10
Moderate	13
Severe	3
Mean SCORAD index (SD)	32.0 (14.99)

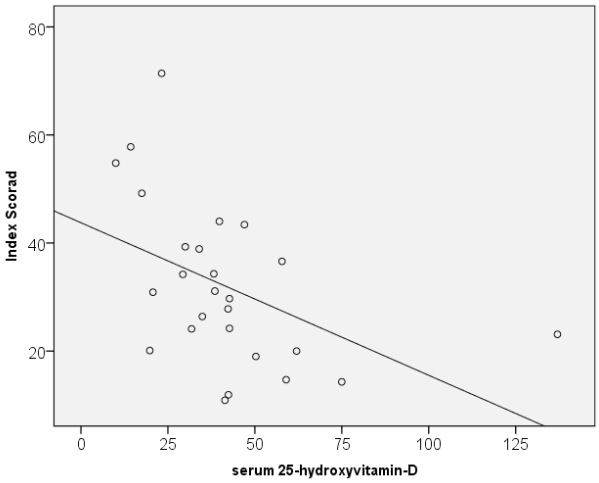

Figure 1. Scatterplot correlation between 25-hydroxyvitamin-D level and SCORAD index

Table 2. Subjects' mean SCORAD index and 25-hydroxyvitamin-D level

	Mean (SD)	Min	Max	95%CI	P value
SCORAD Index	32.0 (14.99)	10.9	71.4	25.95 to 38.06	0.248
25-hydroxyvitamin-D serum, ng/mL	41.6 (24.81)	10	137	31.58 to 51.62	<0.001

Discussion

Atopic dermatitis is a skin disease characterized by an inflammatory reaction on the skin, often due to hereditary and environmental factors.[1] The prevalence of atopic dermatitis in children is 75-80% appearing at first life of age.[2] We found children with AD in the < 1-year-old group was higher than in the 1 to 5-year-old group. The higher prevalence of AD in the first life of age is caused by the failure of immune deviation that should normally select for Th1 cell in immune response skewed to Th2 in post nataly in atopic children.[14]

Atopy is more prevalence in male than female before puberty. There is a reversal of this sex ratio during puberty with girls having more asma and atopy throughout there productive years. Hormonal changes have been implicated in the reversal of sex ratio. Estrogen is understood to have a biphasic dose-response, with higher levels promoting Th2 responses and at lower levels, a Th1 response. Progesterone has been shown to stimulate interleukin-4 production and promote the development of human Th2 cells.15 In this study we found the male to female AD ratio was 1.6:1.

Regarding birth order, the first and the second children were more likely to have atopic dermatitis. In 1989, David Strachan introduced the "hygiene hypothesis," postulating that infection protects against atopy. So reduced exposure to infections during childhood results in aberrant immune responses to innocuous antigens later in life. This hypothesis was based upon Strachan's observations that infants with higher numbers of siblings were at decreased risk for developing allergies.[16,17] In this study we found the first and the second children had more atopic dermatitis.

Familial atopy has been reported to be related to the occurrence of allergic disease manifestation and the severity of AD. A Netherlands study in 1996 reported that of one parent had atopy, the risk of allergic disease in children was 20-40%. If both parents had allergies, then the children had a 60-80% risk. If a sibling had allergic disease, then a child had a risk of 20-30%. And if the family had no history of atopy, then the child's risk of allergic disease was only 10%.[18] Our study found children with AD more prevalence in atopic one of parents than both parents, as seen in **Table 1**. We also found that both atopic parents have all their children with atopic dermatitis. Nine of our subjects had no history of atopy in the family.

It is important to determine the severity of AD in order to evaluate disease improvement during and after therapy. The ETFAD developed the SCORAD index to create a consensus on assessment methods for AD.[5] An Italian study found that lower 25(OH) D level was correlated with higher SCORAD index.[9] Vitamin D plays a crucial role in skin barrier function, where vitamin D3 stimulates the production of cathelicidin.[12] Cathelicidin in macrophages triggers the Th2 response, by reducing dendritic cells maturation and migration, which, in turn, leads to B cells reducing IgE production. Vitamin D also acts as an anti-inflammatory agent; 1,25(OH)D inhibits maturation of dendritic cells and production of cytokines IL-12 and IL-23.[5,8] Cathelicidin is deficient in AD. The pathogenesis of AD involves a complex interplay of epidermal barrier dysfunction and dysregulated immune response, and vitamin D is involved in both processes.[12]

Previous studies have shown that vitamin D can be given as a protective therapy to reduce the severity of AD.[10,19,20] The *American Academy of Pediatrics* (AAP) recommends giving 400 IU of vitamin D as a supplement to newborns to prevent vitamin D deficiency.[5]

In conclusion, there is a moderate correlation between serum 25-hydroxyvitamin-D level and SCORAD index in children with atopic dermatitis.

Conflict of Interest

None declared.

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Original Article

The role of genetic variation in TCF7L2 and KCNJ11, dietary intake, and physical activity on fasting plasma glucagon-like peptide-1 in male adolescents

Harry Freitag Luglio, Emy Huriyati

Abstract

Background Transcription factor 7-like 2 (TCF7L2) and potassium voltage-gated channel subfamily j member 11 (KCNJ11) gene polymorphisms have been associated with type 2 diabetes mellitus (T2DM) via regulation of insulin production. Ingested nutrients induce glucagon-like peptide-1 (GLP-1), which in turn induces insulin secretion.

Objective To evaluate the relationship between TCF7L2 and KCNJ11 gene polymorphism, dietary intake, and physical activity on fasting plasma GLP-1 in normal male adolescents.

Methods This observational study with a cross-sectional design included 54 male adolescents selected from high schools in Yogyakarta, Indonesia. Interviews were done to collect data on energy intake and physical activity. The GLP-1 and insulin levels were measured from fasting blood plasma. The TCF7L2 and KCNJ11 gene polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results Fasting GLP-1 was positively correlated with energy intake ($r=0.276$; $P=0.047$), but not with physical activity ($r=0.011$; $P=0.936$). The GLP-1 concentration was not associated with TCF7L2 and KCNJ11 gene polymorphisms (all $P>0.05$). In subjects with an EE genotype (KCNJ11), GLP-1 was not correlated with insulin ($r=-0.036$; $P=0.435$). However, in subjects with an EK genotype (KCNJ11), GLP-1 was positively correlated with insulin ($r=0.394$; $P=0.026$).

Conclusion GLP-1 concentration is positively correlated with body weight. Among male adolescents with a genetic variation in KCNJ11 (EK genotype), there is a significant correlation between GLP-1 and insulin signalling. **[Paediatr Indones. 2017;57:239-45; doi: http://dx.doi.org/10.14238/pi57.5.2017.239-45]**.

Keywords: *KCNJ11; TCF7L2; GLP-1; diet; physical activity*

Type 2 diabetes mellitus (T2DM) is a disturbance in glucose metabolism as shown by high blood glucose, due to a disorder in insulin secretion or sensitivity.[1] In 2000, the World Health Organization reported that 171 million people worldwide suffered from T2DM.[2] And this disease was responsible for deaths of 1.5 million people in 2012.[3] Epidemiological studies have shown indicators of T2DM in adulthood can be detected from younger ages.[4,5] Insulin resistance and hyperglycemia are good indicators of early onset T2DM, and are prevalent in obese children and adolescents.[6,7]

Environmental and genetic factors have long been associated with insulin resistance in adolescents and the development of T2DM in adults. Dietary factors, such as high energy and fat intake as well as low dietary fiber intake, were shown to be related to increased risk of insulin resistance.[8–10] Obese

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nutritional status increased the risk of insulin resistance; and weight loss was associated with improvement of insulin sensitivity.[11–13]

In addition to environmental factors, several genetic variations that have been associated with increased risk of T2DM. In this study, we aimed to evaluate the role of genetic polymorphism in the TCF7L2 (transcription factor 7-like 2) and KCNJ11 (the potassium inwardly rectifying channel, subfamily J, member 11) genes. Both genes are important in glucose metabolism via regulation of insulin secretion. TCF7L2 gene is located on chromosome 10q25.3 and produces a high-mobility box-containing transcription factor.[14] The TCF7L2 has an important role in the signalling process of glucose metabolism, and variations in this gene have been associated with T2DM.[15] KCNJ11 is a member of the potassium channel gene that is located at 11p15.1 and responsible for production of Kir6.2. Kir6.2 is a component of the ATP-sensitive potassium channel (KATP) in pancreatic beta cells and important in regulation of insulin secretion.[16] Recently, a KCNJ11 gene polymorphism was reported to be associated with increased risk of T2DM.[17]

The TCF7L2 and KCNJ11 genes were associated with T2DM via regulation of insulin production. In pancreatic cells, insulin is released in response to several signals, including glucagon-like peptide-1 (GLP-1). GLP-1 is secreted by enteroendocrine cells (L cells) in the intestinal epithelium, in response to ingested nutrients.[18] GLP-1 induces postprandial insulin secretion and reduces post-prandial hyperglycemia. The effect of GLP-1 as a stimulator of glucose-dependent insulin release has been confirmed by many studies using different approaches.[19-21]

Although the TCF7L2 and KCNJ11 gene products induce insulin secretion from different pathways, they are connected to the regulation of GLP-1. TCF7L2, known as a transcription factor, has an ability to regulate production of GLP-1.[22,23] The KCNJ11 gene produces protein that stimulates insulin secretion via KATP channels. In an animal model, mutation of the KCNJ11 gene induced disturbances of insulin response to the GLP-1 signal.[24]

The role of TCF7L2 and KCNJ11 gene polymorphisms on regulation of GLP-1 production is an interesting connection that is not well understood. GLP-1 may be a good early indicator of T2DM, especially in younger aged children. To our knowledge, no studies to date have highlighted the connection between those genes and GLP-1 production in adolescents. Therefore, we aimed to evaluate the relationship between TCF7L2 and KCNJ11 on fasting plasma GLP-1 levels in adolescents. In addition to genetic factors, we also analyzed for a possible correlation between GLP-1 and lifestyle factors, such as dietary intake and physical activity in male adolescents.

Methods

This observational study with cross-sectional design included normal male adolescents from Yogyakarta, Indonesia. A total of 54 subjects aged 16-18 years were randomly selected from 10 high schools. Those with no medical problems were asked to participate in this study. Subjects were categorized as normal weight, overweight, or obese, based on WHO criteria.[25] This study was approved by the *Medical and Health Research Ethics Committee* (MHREC), Universitas Gadjah Mada Medical School.

Anthropometric measurements were done by trained personnel. Body weight was measured using A digital scale (0.1 kg precision) and height was measured using a microtoise (0.1 cm precision). Neck and waist circumferences were measured using non-stretchable plastic tape. Measurements were done twice, and the means of those measurements were used for further analysis. This study was part of an observational study on genetic, metabolic, and lifestyle aspects of metabolic syndrome in adolescents.[26]

Interviews were conducted to collect data on lifestyle, including dietary intake and physical activity. Dietary intake was measured using a validated, semi-quantitative food frequency questionnaire.[26] Physical activity was measured using an international physical activity questionnaire.[27] Blood collection was done in the morning after 10 hours of fasting. Blood specimens were placed in EDTA tubes and separated into plasma and buffy coat. Plasma was separated from whole blood, then stored at -80°C prior to use. GLP-1 was measured using an enzyme immunoassay (EIA) (*Sigma Aldrich*); insulin was measured using using an enzyme-linked immunoassay (ELISA) (DRG). The DNA sample was isolated from buffy coat using a

DNA isolation kit (Promega). KCNJ11 (Glu23Lys) and TCF7L2 (rs12255372) gene polymorphisms were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), with primers, and restriction enzymes, and conditions shown in **Table 1**.

Table 1. Primers and restriction enzymes used for PCR-RFLP

Genes	Primers (forward)	Primers (reverse)	Restriction enzymes	Incubation temperature & time
KCNJ11 (Glu23Lys)	5'-GACTCTGCAGT-GAGGCCCTA-3'	5'-ACGTTG-CAGTTGCCTTTCTT-3'	5U BAN II	60°C for 3 hours
TCF7L2 (rs12255372)	5'-CTG GAA ACT AAG GCG TGA GG -3'	5'- GGG TCG ATG TTG TTG AGC TT -3'	BseGI	65°C for 3 hours

Statistical analysis was done using *GraphPad Prism version 5.00 for Windows, GraphPad Software*, San Diego, California, USA. Data is presented as mean (standard deviation, SD). Independent T-test was used to compare the anthropometric measures, energy intake, physical activity, GLP-1 level, and insulin level between genotypes. Pearson's correlation test was used to evaluate for possible correlations between plasma GLP-1, insulin level, energy intake, and physical activity. Spearman's correlation test was used to analyze data that were not normally distributed. Results with $P<0.05$ were considered to be statistically significant.

Results

A total of 54 male adolescents were involved in this study. Characteristics of subjects are shown in **Table 2**. Nutritional status was based on BMI for age Z-score. The number of subjects with normal weight (-2<BMI<2), overweight (2<Z-score<3), and obese (Z-score > 3) were 43 (79.63%), 6 (11.11%), and 5 (9.26%), respectively.

The association between TCF7L2 and KCNJ11 gene polymorphisms and anthropometric measures, dietary intake, and endocrine signals are shown in **Table 3**. In TCF7L2 gene polymorphism, no subjects had the TT genotype, hence, the association analysis compared the dominant GG genotype and heterozygote GT genotype. TCF7L2 gene polymorphism was associated with higher body weight, taller height, and greater waist circumference ($P<0.05$). Dietary intake and mean physical activity were not significantly different between those with the GT and the GG genotypes of TCF7L2.

With regards to KCNJ11, all three genotypes were evaluated. We compared the dominant genotype (EE) group to genotypes with the K allele (EK + KK) group, but found no significant associations between KCNJ11 gene polymorphism and anthropometric measures, mean physical activity, or dietary intake.

The fasting plasma insulin and GLP-1 levels were measured in all subjects, and compared between KCNJ11 (EE and EK+KK) and TCF7L2 (GG and GT) genotypes. As shown in **Figure 1**, neither insulin nor GLP-1 significantly differed between KCNJ11 genotypes ($P=0.806$; $P=0.411$, respectively) nor between TCF7L2 genotypes ($P=0.455$; $P=0.531$, respectively).

Table 2. Characteristics of subjects

Characteristics	N=54
Mean age (SD), years	16.3 (0.6)
Mean anthropometric measures (SD)	
Body weight, kg	67.3 (18.7)
Height, cm	168.9 (6.6)
BMI, kg/m^2	23.6 (6.4)
BMI for age Z-score	0.4 (1.6)
Neck circumference, cm	35.4 (5.1)
Waist circumference, cm	80.7 (16.3)
Mean daily dietary intake (SD)	
Energy intake, kcal	3195 (1,097)
Protein intake, g	96.3 (37.2)
Fat intake, g	90.1 (39.8)
Carbohydrate intake, g	483.5 (164.9)
Fiber intake, g	8.9 (4.2)
Mean physical activity (SD), Mets-minute	2397 (1,346)
Mean fasting plasma insulin (SD), µIU/mL	14.8 (10.1)
Mean fasting plasma GLP-1 (SD), pg/mL	61.6 (27.8)

Table 3. The association of TCF7L2 and KCNJ11 gene polymorphisms with anthropometric measurements, dietary intake, and endocrine signals.

Measurements	TCF7L2			KCNJ11		
	GG (n=49)	GT (n=5)	P value	EE (n=23)	EK + KK (n=31)	P value
Mean age (SD), years	16.3 (0.6)	16.4 (0.5)	0.880	16.4 (0.5)	16.3 (0.7)	0.404
Mean anthropometric measures (SD)						
Body weight, kg	65.8 (18.0)	81.9 (20.9)	0.029	69.2 (20.8)	65.8 (17.1)	0.637
Height, cm	168.0 (6.0)	177.6 (6.9)	0.001	168.3 (6.2)	169.4 (7.0)	0.531
BMI	23.4 (6.4)	26.1 (7.1)	0.144	34.5 (7.3)	22.9 (5.7)	0.540
Z score BMI for age	0.3 (1.6)	1.1 (1.4)	0.175	0.6 (1.7)	0.3 (1.5)	0.529
Neck circumference, cm	35.2 (5.3)	36.8 (2.7)	0.071	35.2 (3.3)	35.5 (6.2)	0.751
Waist circumference, cm	78.9 (14.7)	98.4 (21.6)	0.016	81.6 (17.0)	80.1 (16.0)	0.840
Meand daily dietary intake (SD)						
Energy intake, kcal	3,144 (969)	3,697 (2,080)	0.287	3,135 (965)	3,240 (1,199)	0.731
Protein intake, gr	95.3 (34.9)	106.4 (60.0)	0.988	93.8 (35.9)	98.2 (38.7)	0.720
Fat intake, gr	88.5 (37.3)	106.4 (63.1)	0.777	85.6 (40.8)	93.5 (39.4)	0.474
Carbohydrate intake, gr	477.3 (149.6)	543.6 (294.2)	0.397	483.6 (145.1)	483.4 (180.5)	0.523
Fiber intake, gr	8.8 (4.1)	9.4 (5.6)	0.763	8.2 (3.0)	9.4 (4.9)	0.461
Mean physical activity, Mets-minute (SD)	2,372 (1,322)	2,640 (1,720)	0.612	2,630 (1,544)	2,224 (1,176)	0.463

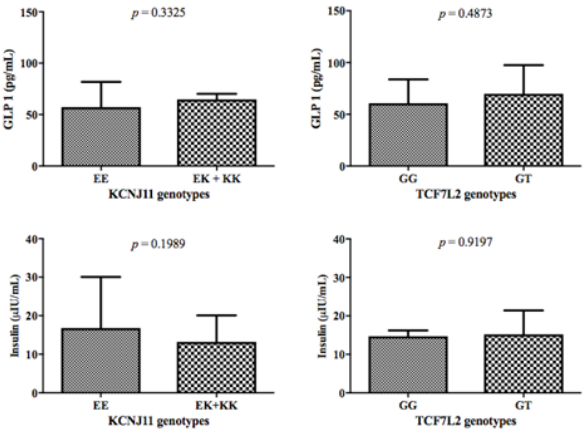

Figure 1. Fasting plasma GLP-1 and insulin levels in genotypes of KCNJ11 and TCF7L2

The possible correlation between insulin and GLP-1 was evaluated in all subjects as well, within the genotype groups. In all subjects, fasting GLP-1 was not associated with fasting insulin level (r=0.063; P=0.326). Subjects were divided by KCNJ11 genotypes, EE and EK. The correlation between insulin and GLP-1 in KK genotypes was not analyzed because the number of subjects is too low. As shown in **Figure 2**, in subjects with the EE genotype, GLP-1 was not correlated with insulin (r=-0.036; P=0.435). However, the EK genotype group had a significant correlation between GLP-1 and insulin (r=0.394; P=0.026). We did not analyze for a possible correlation within TCF7L2 genotypes

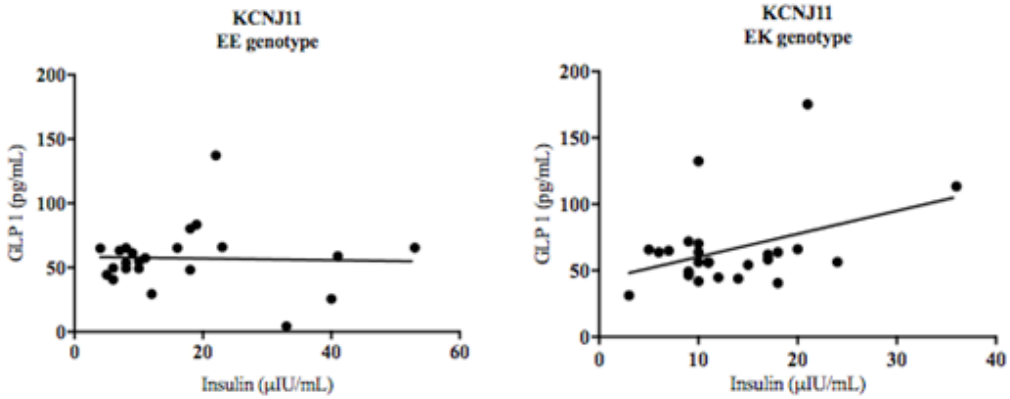

Figure 2. The correlation between fasting insulin and GLP-1 according to KCNJ11 genotypes. In the EE genotype, GLP-1 was not correlated with insulin (r=-0.036; P=0.435) but in the EK genotype, GLP-1 had a significant, positive correlation with insulin (r=0.394; P=0.026).

because of the small number of subjects in one of the genotype groups.

In order to evaluate lifestyle factors, we analyzed the relationship between energy intake, physical activity, and fasting plasma GLP-1. Total energy intake had a positive, significant association with GLP-1, after controlling for weight and age (r=0.276; P=0.047). However, there was no significant correlation between physical activity and GLP-1 (r=0.011; P=0.936) (**Figure 3**). Body weight was also significantly correlated with fasting GLP-1 level (age controlled, r=0.444; P=0.001).

metabolism.[27] Postprandial GLP-1 secretion was reported to be lower in T2DM patients than those without T2DM, and administration of a GLP-1 analogue improved glucose control in T2DM patients.[28,29] Because GLP-1 induces insulin secretion, it has been argued that the increasing GLP-1 production might lead to hyperinsulinemia and insulin resistance.

Growing evidence has shown that GLP-1 production is an early indication of metabolic syndrome. Munoz *et al.* showed that obese individuals had higher fasting GLP-1 concentration and also

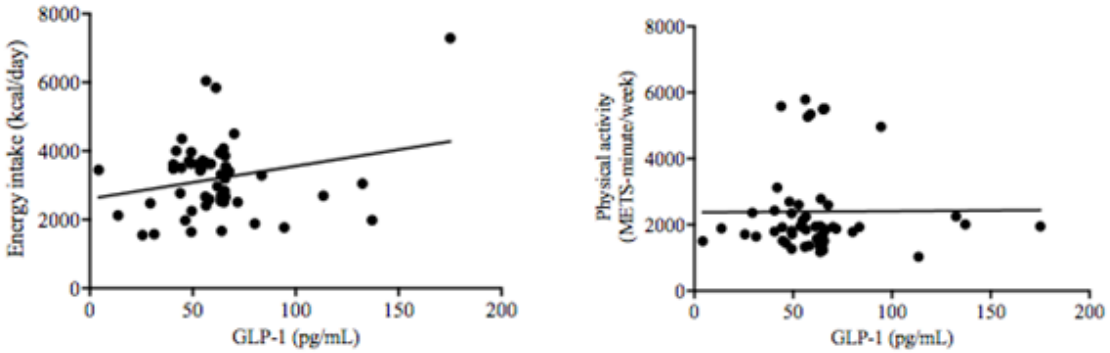

Figure 3. The correlation between fasting plasma GLP-1 on energy intake (r=0,276; P=0.047) and physical activity (r=0.011; P=0.936), by Pearson's correlation analysis (2-tailed, corrected for body weight and age).

Discussion

In this study, we evaluated factors for possible associations with GLP-1 production in male adolescents, including lifestyle and genetic factors. With regards to lifestyle, fasting GLP-1 production was positively correlated with total energy intake, but not with physical activity. With regards to genetics, fasting GLP-1 concentration was not associated with TCF7L2 or KCNJ11 gene polymorphisms. However, the correlation between fasting GLP-1 and insulin level was dependent on genetic background. In subjects with the EE genotype of KCNJ11, GLP-1 was not associated with insulin. However, those with the EK genotype of KCNJ11 had a positive correlation between GLP-1 and insulin.

Because of its insulinotropic effect, GLP-1 production has been associated with better glucose disturbed diurnal GLP-1 variation.[30] Additionally, Yamaoka-Tojo *et al.* reported that a peripheral GLP-1 concentration was positively correlated with metabolic syndrome. They suggested that the conditions of overnutrition and obesity increased GLP-1 production and lead to GLP-1 dysfunction.[31]

Those ideas were supported in this study. We showed that body weight was positively correlated with fasting GLP-1. Additionally, energy intake was also correlated with fasting GLP-1, independent of body weight and age. This showed that habitual energy intake was correlated with fasting GLP-1 production our subjects. In all subjects, we showed that GLP-1 was not correlated with insulin concentration. However, after analyzing by genetic profile, we observed a positive correlation between GLP-1 and insulin level in subjects with the EK genotype of KCNJ11 gene. Interestingly, this correlation was not seen in the EE genotype of KCNJ11 gene. These results are evidence of the importance of KCNJ11 gene polymorphism on

the insulinotropic effect of GLP-1.

The interaction between KCNJ11 and GLP-1 action has been shown in an animal trial. Hugill *et al.* induced a KCNJ11 point mutation in mice that induced impaired glucose tolerance and defective insulin secretion. Additionally, they showed that mice with KCNJ11 gene mutation had an impaired response to GLP-1 and glucose-dependent insulinotropic peptide (GIP).[24] To date, the mechanism of how the genetic mutation in the KCNJ11 gene affects GLP-1 production or the insulin response to GLP-1 remains unknown. As such, this study is the first to assess genetic variation in KCNJ11 and the insulin response to GLP-1 production. While our study was observational, had a small number of subjects, and assessed blood specimens taken in a fasting state, our results provide a basis for further study on the physiological effect of KCNJ11 on GLP-1 and insulin regulation.

TCF7L2 and KCNJ11 genes are important in regulating insulin release and genetic variation of those genes were associated with T2DM15-17. In this study, we analyzed the association of those genetic polymorphisms with GLP-1 and insulin concentration in male adolescents. Analyzing the correlation between GLP-1 and insulin was necessary because suspect that GLP-1 and insulin can be used as an early indicator of metabolic disturbance in glucose metabolism. However, in this study we did not find a significant difference in GLP-1 and insulin level among those with TCF7L2 and KCNJ11 gene variations.

In this study, we also showed that TCF7L2 gene polymorphism was associated with higher body weight, taller height, and greater waist circumference. Subjects with T allele of those gene variation has more body weight and waist circumference than those without T allele. However, because the number of subjects in GT group was very low (only 5 subjects), it is very important to be careful when generate conclusion based on this finding.

In conclusion, GLP-1 and insulin have a significant association in male adolescents with the EK genotype of KCNJ11, perhaps shedding more light on the association between KCNJ11 gene variation and insulin resistance as well as T2DM. Additionally, GLP-1 is associated with nutritional status and energy intake. Because this study involved only a small number of subjects and exclusively male adolescents, we suggest further study on a broader population.

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Conflict of Interest

None declared.

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Original Article

Effect of community-based food supplementation on improving growth of underweight children under five years of age in West Nusa Tenggara

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Abstract

Background The prevalence of underweight children in West Nusa Tenggara is as high as 30%. This region had the third largest number of stunted children in the country. The local government has attempted to tackle this problem by providing supplementary food to underweight children.

Objective To assess the success of the community-based food supplementation program on improving children's growth in West Nusa Tenggara.

Methods We conducted a prospective cohort study for 10 months in Paruga District Primary Health Care Unit, Bima, West Nusa Tenggara, in year 2012. Children were given supplementary food according to the Ministry of Health's guidelines, consisting of formula milk, high calorie biscuits, and a 60-day supply of eggs, estimated to be sufficient to normalize their weights, for their age and sex. A child's weight and height were measured every 3 months and the results plotted on WHO growth charts for weight-for-age, height-for-age, and weight-for-height (nutritional status). Z-score <-3 SD was classified as severely underweight, severely stunted, or severely wasted, respectively; Z-score between -2 and -3 SD was classified as underweight, stunted, or wasted, respectively; and Z–score >-2 SD was classified as normal for all three categories.

Results Twenty-five children under five years of age participated in this study. Subjects' median age was 29 months. None of the subjects had normal weight-for-age Z-score at the beginning of the study. Eighty-four percent (21/25) of the subjects were severely underweight. Only 8% (2/25) of the subjects had normal height-for-age Z-score and 88% (22/25) of them were severely stunted. However, 80% (20/25) of subjects had normal nutritional status (weight-for-height). Changes in weight-for-age Z-score varied throughout the study. The highest median score was in the tenth month of follow up (-3.82). The highest median height-for-age score and weight-for-height score were also in the last month of follow up. At the end of the study, only one subject had normal weight-for-age score (4%) and none of the subjects had normal height-for-age scores.

Conclusion The 10-month supplementary food program for under-five children in the Paruga District is not successful in improving body weight and height. **[Paediatr Indones. 2017;57:246-51; doi: http://dx.doi.org/10.14238/pi57.5.2017.246-51]**.

Keywords: *under-five children; stunting; nutritional status; community*

Stunting in children remains a global health problem.[1,2] Globally, about 1 in 4 children under 5 year-old are stunted (26% in 2011). An estimated 80% of the world's 165 million stunted children live in just 14 countries. *The World Health Assembly* has adopted a new target of reducing the number of stunted children under the age of 5 by 40% by 2025.[2] Furthermore, it has also been decided that the new target in the *Sustainable Development Goals* (SDGs) is to end stunting and wasting in

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children under-five by 2025.[3] In 2013, Indonesia ranked fifth largest in the number of stunted children and fourth largest for the number of underweight children, in a worldwide comparison.[1] The prevalence of underweight children in West Nusa Tenggara has been as high as 30%, and the province was ranked third highest in the number of stunted children in Indonesia.[4] However, Indonesian studies on stunting and its treatment have been limited in number.

The local government, through its primary health care units, has tried to tackle this nutrition problem. One of the programs they proposed was to give supplementary food to children whose weights were below the red line, according to the health card (*Kartu Menuju Sehat, KMS*). We aimed to evaluate the success of this intervention on improving the nutritional status of children under-five years of age in Bima, West Nusa Tenggara.

Methods

We conducted a prospective cohort study in the Paruga District Primary Health Care (PHC) unit, Bima, West Nusa Tenggara. Our subjects were children under five years of age whose weights were below the red line, according to the KMS curve in January 2012. The children were given supplementary food according to Ministry of Health guidelines, consisting of milk formula, high calorie biscuits, and a 60-day supply of eggs. Parents picked up the supplement from the PHC unit and were instructed to give the supplementary food to their children every day, according to the PHC personnel instructions. These supplementary foods were given until the child's weight was above the red line.

A child's weight and height was measured every 3 months and the results plotted on *WHO* growth charts for weight-for-age, height-for-age, and weight-for-height. Z-score <-3 SD was classified as severely underweight, severely stunted, or severely wasted, respectively; Z-score between -2 and -3 SD was classified as underweight, stunted, or wasted, respectively; and Z–score >-2 SD was classified as normal for all three categories. Z-score was calculated using the *WHO Anthro Application* to acquire a raw score data.

We used a consecutive sampling method to include subjects under-five years whose weights were below the red line on the *KMS* curve in January 2012. However, subjects with poor compliance were excluded from the study. Poor compliance was described as not attending one or more visit for measurement.

Nominal data are presented in numbers of case (percentage), while continuous data are presented in median (minimum-maximum). Changes in Z-score for weight-for-age, height-for-age, and weight-for-height in the first, fourth, seventh, and tenth month were analyzed using Wilcoxon signed rank test. Statistical analysis in this study was performed using *SPSS 19.0 for Windows* software. This study was approved by the Ethics Committee of Dr. Cipto Mangukusumo Hospital, Jakarta, linked to the University of Indonesia, Jakarta.

Results

There were 31 under-five children whose weights were below the red line on the KMS curve in January 2012. Six children were excluded due to poor compliance. A total of 25 children (12 boys and 13 girls) were accepted as study subjects. Subjects' median age was 29 months, ranging from 12 to 54 months. Subjects' initial median length/height in January 2012 was 77 cm, while median weight was 8.8 kg. The baseline characteristics of subjects are summarized in **Table 1**.

In January 2012, subjects' Z-scores for weight-for-age (WAZ) were -4.56 to -2.50, with median -3.35 (**Figure 1**). Three months later in April 2012, the WAZ range was probably not improved (-4.52 to -2.59). At the next three-month follow-up in July 2012, the median WAZ increased to -3.15, and the range was -4.83 to -2.30. In the last follow-up (October 2012), the median WAZ was the highest (-3.14), but unfortunately, there was a bigger discrepancy between the minimum and maximum values (-6.39 to -1.99).

In January 2012, subjects' Z-score for height-for-age (HAZ) were -6.33 to -1.66, with a median of -4.49 (**Figure 2**). The scores were even lower in April 2012 (-6.91 to -2.40), but showed slight improvement in July 2012 (-5.72 to -2.63). At the end of the study in October 2012, the HAZ showed very little improvement (-6.12 to -3.05) compared to the Z-score in January. Friedman test detected a

Table 1. Baseline characteristics of subjects

Characteristics	N=25
Gender, n	
Male	12
Female	13
Median age (range), months	29 (12-54)
Median initial weight (range), kg	8.3 (6.2-10.6)
Median initial length/height (range), cm	77.0 (60.0-84.0)
Weight (weight-for-age), n	
Normal (Z-score > -2SD)	0
Underweight (Z-score between -2 and -3 SD)	4
Severely underweight (Z-score <-3SD)	21
Stature (height-for-age), n	
Normal (Z-score >-2 SD)	2
Stunted (Z-score between -2 and -3 SD)	1
Severely stunted (Z-score <-3 SD)	22
Nutritional status (weight-for-height), n	
Normal (Z-score > -2 SD)	20
Wasted (Z-score between -2 and -3 SD)	4
Severely wasted (Z-score <-3 SD)	1

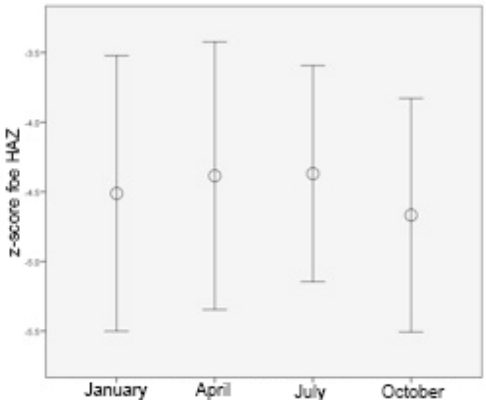

Figure 2. Height-for-age Z-score changes during 10 months of follow-up
Note: Dots upon box and whisker plot represent median value

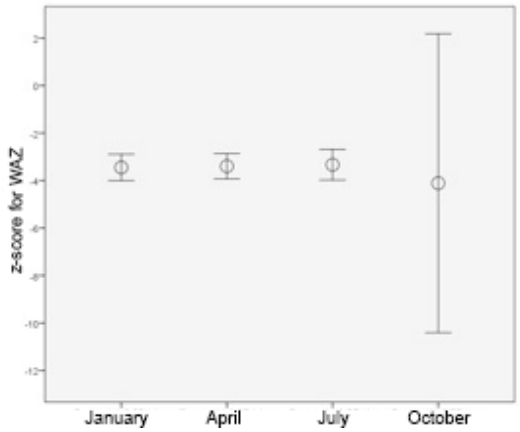

Figure 1. Weight-for-age Z-score changes during 10 months of follow-up
Note: Dots upon box and whisker plot represent median value

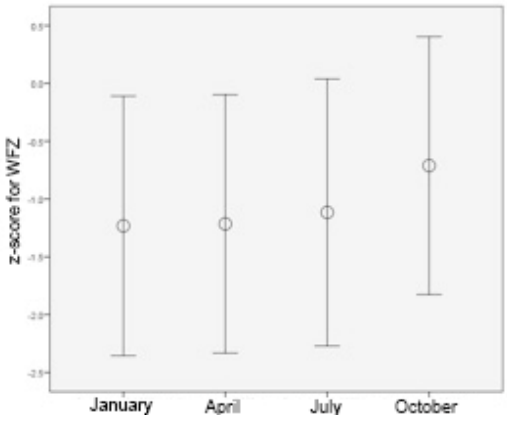

Figure 3. Weight-for-height changes during ten months of follow-up
Note: Dots upon box and whisker plot represent median value

significant difference in the months that body height measurement was done. However, Wilcoxon signed rank test revealed that only the changes between April to October 2012 (P=0.001) and July to October 2012 (P<0.001) were statistically significant. Changes in HAZ throughout the study are depicted in **Figure 2**.

As seen in **Figure 3**, the median weight-for-height Z-score (WFZ) was within normal limits (-1.40) at the beginning of our study (range -3.14 to -1.03). Three months after intervention, the median WFZ slightly decreased to -1.42 (-3.31 to -1.38). In the sixth month of follow-up, there was improvement, with median WFZ -1.11 (-4.14 to -1.01). The highest median WFZ was achieved in the tenth month of intervention [-0.70 (-3.22 to -1.63)].

Discussion

Growth stunting can be used as an indicator of nutritional status in children.[2] Moreover, stunting has diverted attention from underweight status in children,[5] and is important since it may have a deleterious impact on cognitive level and work performance.[1,2,5] Several risk factors contribute to stunting in children: nutrition, genetics, and endocrine factors.[2,5] In the first two years of life, a child's growth is very dependent to nutritional factors, thus, at that age, food security with balanced nutrition should be optimized.[5-7]

Stunting is defined as height-for-age <-2 SD for moderate, and <-3 SD for severe stunting, in children aged 0 to 59 months, from the median 2006 WHO Child Growth Standards. Stunting is caused by malnutrition. Growth faltering is defined by any decline in linear growth that crosses 2 percentiles in the growth chart.

According to UNICEF, undernutrition is an effect caused by two basic problems: illness and inadequate food intake. Those two basic problems result from lack of clean water and sanitation, household food insecurity, and/or lack of health care facilities.[2] Our subjects were underweight from the beginning of the study, as reflected by their low WAZ. This condition was probably due to all of the causes mentioned above.

Body weight was not dramatically increased throughout the study, as shown by number of subjects who had normal weight-for-age Z-scores. At the end of our study, only one subject had WAZ >-2 SD. This slow progression might have been caused by inadequate food intake, infection, HIV/AIDS, and/or psychological problems. Intervention by the PHC was considered to be adequate in quality and quantity. The food supplementation consisted of eggs, high calorie biscuits, and milk formula. However, we did not monitor how the parents gave the supplementary food to the children day by day. The presence of illness and psychological problems also were not noted in our study.

A similar study was conducted in Senegal in under-three children. The study found increased body weight and reduced number of malnutrition cases. Interventions provided were body weight measurement, as well as supplementation of vitamin A and iron. The Senegal study also found that children born from a mother who received nutritional education during pregnancy had better nutritional status than those born to mothers who did not receive nutritional education.[8] As such, we recommend that to our PHC officers add maternal education to their routine program.

We also observed that our subjects had low HAZ: 4% (1/25) stunted and 88% (22/25) severely stunted. Waterlow and Schurch stated that one etiology of stunting is chronic malnutrition.[9] According to Li *et al.* failure to treat acute malnutrition during pregnancy and through the first two years of life will lead to stunting.[10] If the child is already two years old, like most of our subjects, catch-up growth could occur during puberty or a secondary growth spurt. However, a systematic review by Dewey *et al.*[11] showed that supplementary feeding did not improve height-for-age Z-score.[12]

Body height data is very important for determining a child's nutritional status. A mere use of body weight data is not reliable since it is easily influenced by many factors. Our subjects were diagnosed as underweight (Z-score between -2 and -3SD), thus they were given supplementary food for 60 days until their weight was considered normal according to their current age and sex. If body height was taken into account in determining their nutritional status, 20 subjects (80%) had good nutritional status. However, only one of them had normal body height, according to age and sex.

Z-scores were slightly lower when measured using the WHO standard (**Figure 2**). The CDC chart shows shorter children, compared to the WHO chart.8 Therefore, the WHO chart yields lower Z-scores, thus explaining the higher stunting rate based on the WHO standard. Although the Z-score results were different, the interpretation of the data still showed that subjects were stunted, by either chart. Another disadvantage of the WHO standard is that the chart increases the disease burden up to two-fold, compared to the CDC standard.[8]

The supplementary food program was unsuccessful in improving our subjects' growth. **Figures 1** and **2** show that both median weight and height did not improve during the 10 months of follow-up. Although some changes between the months were statistically significant, the finding is clinically important since

it indicates that our subjects failed to catch up to normal growth.

Nutrition-specific intervention recommended by UNICEF includes maternal nutrition therapy, in order to reduce low birth weight in newborns.[2,3] Low birth weight babies tend to be stunted in adult life.[3,5] Other recommendations are promotion of exclusive breastfeeding, introduction of supplementary food starting from 6 months of age, micronutrient supplementation, sanitation, and access to healthcare providers.[3,6] This nutrition-specific therapy should be given from pregnancy through the first 2 years of life, but is not necessary afterwards.[3] Bhutta *et al.* reported that food supplementation with or without maternal education could improve height-for-age Z-scores up to 0.41 points (0.05-0.76).[13] Their intervention successfully reduced stunting due to malnutrition by 36% for children under-three. We did not provide maternal nutritional education, so this recommendation should be taken into consideration. We recommend training of medical personnel who work in rural areas to use the appropriate chart, in order to analyze the growth trend in one specific area. Serial measurement of body height and weight for every child in one district is mandatory. All data should be carefully recorded to allow for comparison to prior and future data, so we can assess the nutritional status of the area's population.

Based on above results, food supplementation was not successful in improving children's growth. Several possible contributing factors could include household food insecurity, poverty, chronic disease, as well as poor hygiene and sanitation.[5,6] Those factors were not examined in this study. In addition, a child's potential height should be determined to assess whether the child is growing according to his genetic potential.

Yet another reason for the failure of the food program, was that our subjects' short stature may not have been due to malnutrition. Normal nutritional status (WHZ >-2SD) was observed in 80% of subjects, so chronic malnutrition was not the cause of our subjects' short stature. Our subjects may have originally had other problems, not undernutrition. Normal genetic variations should be considered. Unfortunately, data on our subjects' body height since birth were not available. Since our subjects were probably short due to factors other than malnutrition such as history of low birth weight or genetic predisposition, the recommendations we can draw from this study are to measure children's height and weight regularly every 3-6 months, and to assess other confounding variables. Physicians then can determine whether the child is short due to genetic variations or other pathologic conditions, before deciding to give any nutritional intervention. The weaknesses of our study were the small sample size, short duration of observation, as well as a lack of data on birth weight, birth length, and parents' heights. Furthermore, future studies should include close monitoring on how the supplementary food is being optimally given to the needing child.

In conclusion, the 10-month supplementary food program given is not successful in improving body weight and height of children under-five in Paruga District. Most of our subjects are short due to factors other than malnutrition, and 80% of the subjects have normal nutritional status since the beginning of the study. Our main recommendation from this study is to measure body height regularly and ensure that all supplementary food is successfully delivered only for the underweight child.

Conflict of Interest

None declared.

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Original Article

Upper arm fat and muscle in stunted and non-stunted children aged 0-24 months

Bagus Winandi Arundito, Madarina Julia, Neti Nurani, Endy P. Prawirohartono

Abstract

Background The prevalence of stunting in Indonesia is high, with particularly negative impacts on health during childhood as well as adolescence. Stunting impacts the health of children as well as adults, especially with regards to future obesity. Therefore, evaluating body composition of stunted children before 2 years of age is necessary.

Objective To compare upper arm fat and muscle measurements in stunted and non-stunted children aged 0-24 months of age.

Methods We analyzed secondary data of the Division of Nutrition and Metabolic Disease, Department of Child Health, Universitas Gadjah Mada Medical School, Yogyakarta which were collected using cluster random sampling from the Yogyakarta Special Province. We compared upper arm fat area (UFA), including the upper arm fat area estimate (UFE) and the upper arm fat percentage (UFP), as well as upper arm muscle area (UMA) and upper arm muscle area estimate (UME), among stunted and non-stunted children aged 0-24 months.

Results We analyzed 2,195 children. The prevalence of stunting was 354/2,195 (16.1%). The UFA, UFE, and UFP among stunted children were significantly lower compared to non-stunted children aged 7-12 months [UFA: 4.48 *vs.* 5.05 cm2 (P <0.001), respectively; UFE: 4.88 *vs.* 5.55 cm2 (P <0.001), respectively; and UFP: 30.82 *vs.* 32.58% (P = 0.03), respectively]. The UMA in children aged 7-12 months was also significantly lower in stunted than in non-stunted children [11.31 *vs.* 11.79 cm2 (P = 0.02), respectively], as well as in children aged 13-24 months [11.05 *vs.* 11.75 cm2 (P <0.001), respectively]. In addition, the UME in children aged 13-24 months was significantly lower in stunted compared to non-stunted children [10.50 *vs.* 11.18 cm2 (P <0.001), respectively].

Conclusion The UFA in children aged 7-12 months is smaller in stunted than in non-stunted children, whereas UMA in children aged 7-12 months and 13-24 months was smaller in stunted compared to non-stunted children. **[Paediatr Indones. 2017;57:252-61; doi: http://dx.doi.org/10.14238/pi57.5.2017.252-61]**.

Keywords: *stunting; overweight; obesity; upper arm fat area; upper arm muscle area; upper arm fat percentage*

Prevalence of stunting was high in developing countries (39.7% in 1990), but predicted to decrease to 21.8% in 2020.[1] In Indonesia in 2013 the prevalence was 37.2%.[2] Stunting impacts the health of children as well as adults, especially with regards to future obesity.[3] Therefore, evaluating body composition of stunted children before 2 years of age is necessary.

Previous studies have shown that upper arm anthropometry, i.e., fat mass measured as upper arm fat area (UFA) and upper arm fat area estimate (UFE), as well as muscle mass measured as upper arm muscle area (UMA) and upper arm muscle

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estimate (UME) could be representive of a child's body fat and muscle mass.[4-5] The simple methods of upper arm anthropometry to assess fat and muscle mass are clinically beneficial, since the standard methods of assessing body composition, i.e., dual X-ray absorptiometry, isotope dilution, hydrostatic weighing, bioelectrical impedance analysis, air displacement plethysmography, and total body electrical conductivity[6] are not generally available in primary health facilities.

Unfortunately, few studies have been done on fat and muscle mass of stunted compared with non-stunted children less than 24 months of age in Indonesia. Studies on the significance of upper arm anthropometry as representative of the child's body composition are important as a practical method of promoting health in primary care settings. Stunted children less than 2 years of age should be monitored regularly to identify increased fat, using upper arm anthropometry. Children usually experience stunting during the first 2-3 years, and they will have difficulty catching up their growth if they remain in a poor environment.[7]

The aim of the study was to evaluate fat and muscle mass, measured as UFA, UFE, UMA, UME, and UFP, in stunted compared with non-stunted children 0 to 24 months of age in Yogyakarta Special Province, Indonesia.

Methods

We analyzed secondary data from the Division of Nutrition and Metabolic Disease, Department of Child Health, Universitas Gadjah Mada Medical School, Yogyakarta. The study population was children 0 to 24 months of age from Yogyakarta Special Province who were recruited using a multi-stage cluster sampling method from the primary health center (posyandu) as the sampling unit. We randomly selected 6 posyandu (351 children) from Yogyakarta Municipality, 10 posyandu (573 children) from Bantul Regency, 7 posyandu (688 children) from Sleman Regency, 7 posyandu (338 children) from Gunung Kidul Regency, and 7 posyandu (255 children) from Kulon Progo Regency, for a total of 37 posyandu (2,205 children) .

Children who fulfilled the inclusion criteria were 0 to 24 months of age and had mothers who consented to participate. We excluded children who were ill at the time of data collection. Anthropometric data [weight, length, middle upper arm circumference (MUAC), and triceps skinfold thickness (TS)] were measured. Demographic data of the family included parental education and occupation, number of children, and child's birth weight. We added exclusion criteria for data analysis when anthropometric data of the registry were missing.

Weight, length, MUAC, and TS were measured by a trained research assistant. All measurements were taken in triplicate and mean values calculated. Children were weighed using a *GEA®* Baby Scale, and length was measured using a locally produced wooden board. *Harpenden Skinfold Caliper®* (*Baty International RH15 9LR* England) was used to measure TS and an upper arm measuring tape (Indonesian Ministry of Health) was used to measure MUAC. Measurement techniques were based on standardized methods.[8-9]

The minimum required sample size was calculated for two unpaired samples with nominal scale of dependent variable and estimating a difference of two proportion.[10] We assumed that the value of $\alpha = 1.960$, proportion of overweight and/or obese among stunted children from literature was 0.1,[11] proportion of overweight and/or obesity among stunted children was 0.075 (researcher's judgment calculated from secondary data), and absolute precision 0.05 with 95% confidence interval of 0.05. Therefore, the minimum required sample size was 245 children.

We defined stunting to be height-for-age z-score (HAZ) < - 2 SD, based on the 2006 *WHO Child Growth Standard*.[12] A child with weight-for-age z-score (WHZ) > 2 SD was classified as overweight or obese. The UMA, UFA, and total upper arm area (TUA) were calculated using the following formulas: UMA = [MUAC – (TS x π)]2 / (4 π) (cm2); UFA = TUA – UMA (cm2); and TUA = MUAC2 / 4π (cm2).4 The UFE and UME were calculated using the following formulas: UFE = MUAC x (TS/2) (cm2) and UME = TUA – UFE (cm2). We included UFE and UME instead of UFA and UMA, since both UFE and UME were well correlated with MRI which precisely measured fat as well as muscle areas.5 Upper arm fat percentage (UFP) was calculated by the formula UFE x TUA x 100%.[4,5]

We analyzed the prevalence difference of

overweight or obesity between stunted and non-stunted children according to age groups by Chi-square test. The mean differences of UFA, UFE, UMA, UME and UFP between stunted and non-stunted children were tested using unpaired T-test. We set the statistical significance at $P < 0.05$, and analyzed data using the *Statistical Package for the Social Science (SPSS) version 15.0* (*SPSS Inc.*, Chicago, IL, USA) software. This study was approved by the Research and Health Ethics Committee of Gadjah Mada University Medical School/Dr. Sardjito Hospital, Yogyakarta, Indonesia.

Results

Figure 1 shows the trial profiles. The highest prevalences of stunting in girls (42%) and boys (52%) were at 22 and 21 months of age, respectively (**Figure 2**). **Table 1** shows the basic characteristics of study subjects, where subjects were grouped by age (0-6, 7-12, and 13-24 months). **Table 2** indicates that in total, the prevalence of overweight or obesity in non-stunted children was significantly higher compared to stunted children (72.5% *vs.* 27.5%, respectively, P=0.005). The prevalences of overweight or obesity in non-stunted compared to stunted children were significantly different in two age groups: 0-6 months (5.38% *vs.* 17.65%, respectively, P=0.001), and 7-12 months (2.81% *vs.* 9.45%, respectively, P=0.002).

Figure 2. The proportion (%) of stunting (indicated by numbers on rows of boys and girls) according to age (0-24 months) among boys and girls.

Stunted children aged 7-12 months had significantly lower UFA, UFE, and UFP means than non-stunted children of the same age group [UFA: 4.48 *vs.* 5.05 cm^2, respectively, (P<0.001) (**Table 3**); UFE: 4.88 *vs.* 5.55 cm^2, respectively, (P<0.001) (**Table 4**); and UFP: 30.82 *vs.* 32.58% (**Table 5**), respectively, (P=0.03)].

We created scatter plots to identify trends in UFA, UFE, and UFP from birth to 24 months of age.

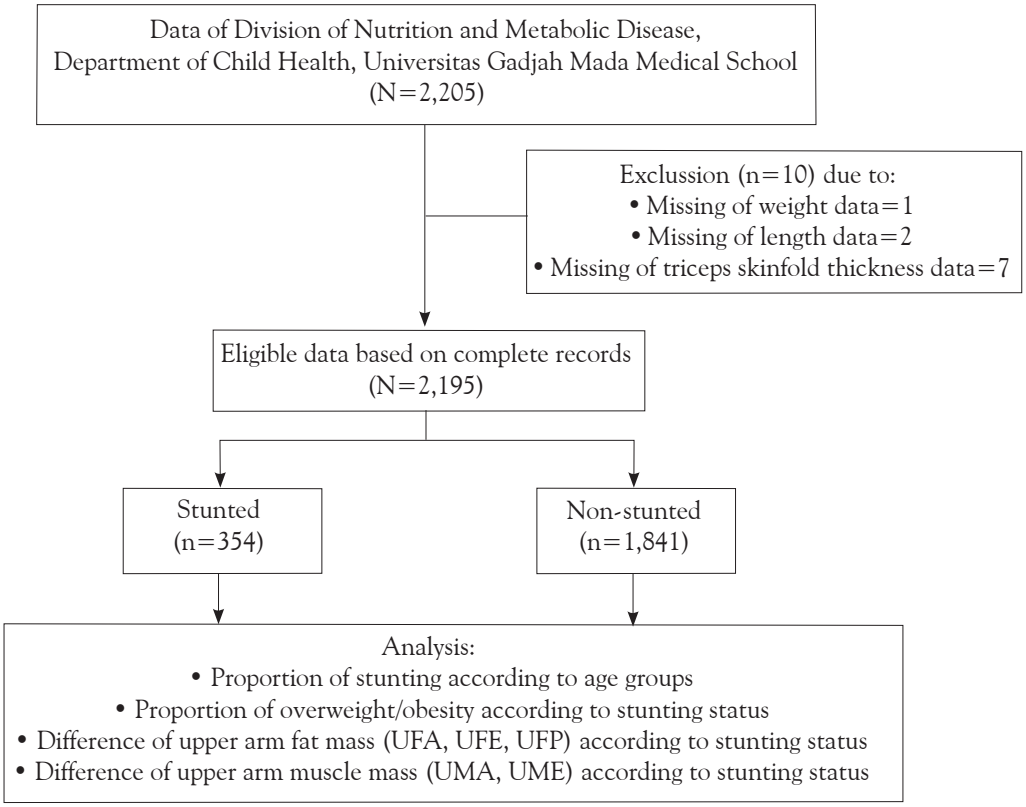

Figure 1. Trial profile

Table 1. Basic characteristics of subjects (N=2,195)

Characteristics	n(%)
Gender	
Male	1,137 (51.8)
Female	1,058 (48.2)
Age groups	
0-6 months	885 (40.3)
7-12 months	677 (30.8)
13-24 months	633 (28.8)
Number of children in family	
1	1,040 (47/4)
2	706 (32.2
3	297 (13.5)
4	94 (4.3)
>4	55 (2.5)
Stunted	
Yes	354 (16)
No	1,841 (84)

Figures 3, 4, and **5** show that mean UFA, UFE, and UFP appeared lower in stunted children aged 4-12 months, 4-12 months, and 8-11 months, respectively, compared to non-stunted children of the same respective age groups.

Non-stunted children aged 7-12 months and 13-24 months also had significantly higher mean UMA compared to stunted children of the same age groups, where the mean differences were 0.48 cm^2 (P=0.02) and 0.70 cm^2 (P< 0.001) (**Table 6**) respectively. Mean UME in the 13-24 month age group was also significantly higher in non-stunted children than in stunted children, with a mean difference of 0.68 cm^2 (P< 0.001) (**Table 7**).

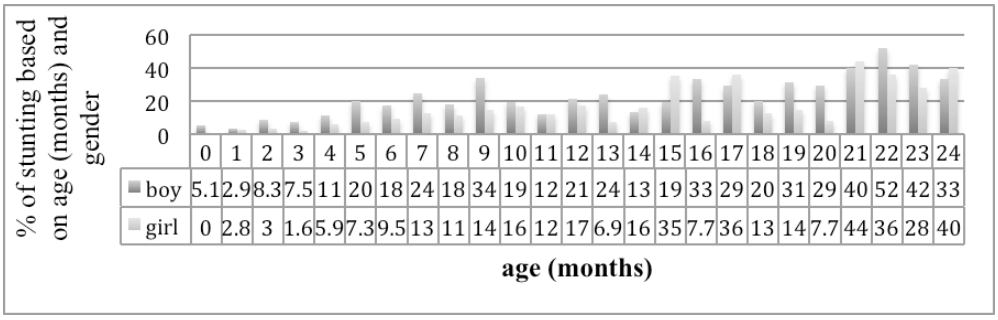

Figure 2. The proportion (%) of stunting (indicated by numbers on rows of boys and girls) according to age (0-24 months) among boys and girls

Table 2. Prevalence of overweight or obesity among non-stunted and stunted children according to age groups

Age groups and overweight/obesity status	Stunted	Non-stunted	P value
0-6 months, n (%)			
Overweight/obese	12 (21.8)	43 (78.2)	0.005
Not overweight/obese	54 (6.5)	776 (93.5)	
7-12 months, n (%)			
Overweight/obese	12 (46.2)	14 (53.8)	0.001
Not overweight/obese	112 (17.2)	539 (82.8)	
13-24 months, n (%)			
Overweight/obese	1 (10)	9 (90)	0.446
Not overweight/obese	163 (26.2)	460 (73.8)	
Total, n (%)			
Overweight/obese	25 (27.5)	66 (72.5)	0.005
Not overweight/obese	329 (27.5)	1,775 (84.4)	

Table 3. Mean UFA in stunted and non-stunted children according to age groups

Age groups	Stunted		Non-stunted		Mean difference, cm^2	95%CI	P value
	Mean UFA, cm^2	SD	Mean UFA, cm^2	SD			
0-6 mo	4.84	1.38	4.79	1.30	-0.05	-0.38 to 0.28	0.76
7-12 mo	4.48	1.36	5.05	1.73	0.57	0.29 to 0.85	<0.001
13-24 mo	5.26	1.70	5.45	2.04	0.19	-0.16 to 0.54	0.28
Total	4.91	1.56	5.04	1.66	0.13	-0.06 to 0.32	0.17

Table 4. Mean UFE in stunted and non-stunted children according to age groups

Age groups	Stunted		Non-stunted		Mean difference, cm^2	95%CI	P value
	Mean UFE, cm^2	SD	Mean UFE, cm2	SD			
0-6 mo	5.36	1.62	5.29	1.49	-0.07	-0.45 to 0.31	0.72
7-12 mo	4.88	1.58	5.55	2.03	0.67	0.36 to 0.99	<0.001
13-24 mo	5.81	2.01	6.01	2.41	0.20	-0.17 to 0.59	0.28
Total	5.40	1.84	5.56	1.95	0.16	-0.07 to0.37	0.18

Table 5. Mean UFP between stunted and non-stunted children according to age groups

Age groups	Stunted		Non-stunted		Mean difference, cm^2	95%CI	P value
	Mean UFP, cm^2	SD	Mean UFP, cm^2	SD			
0-6 mo	36.53	9.21	36.47	7.16	-0.06	-1.90 to 1.78	0.95
7-12 mo	30.82	7.78	32.58	9.08	1.76	0.19 to 3.34	0.03
13-24 mo	35.10	9.00	34.28	10.03	-0.82	-2.57 to 0.92	0.35
Total	33.87	8.91	34.74	8.71	0.87	-0.12 to 1.87	0.08

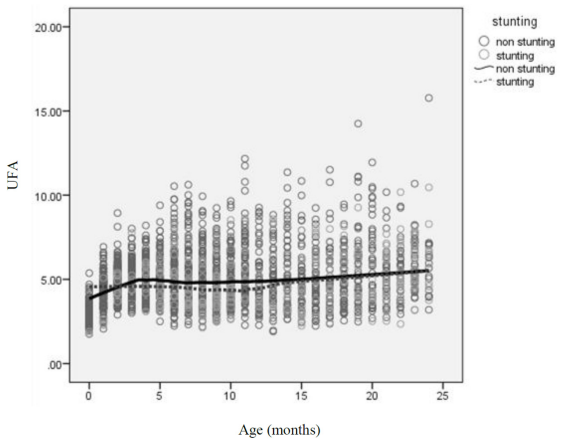

Figure 3. Scatter plot for UFA of stunted and non-stunted children according to age in months, showing the trend from birth to 24 months of age

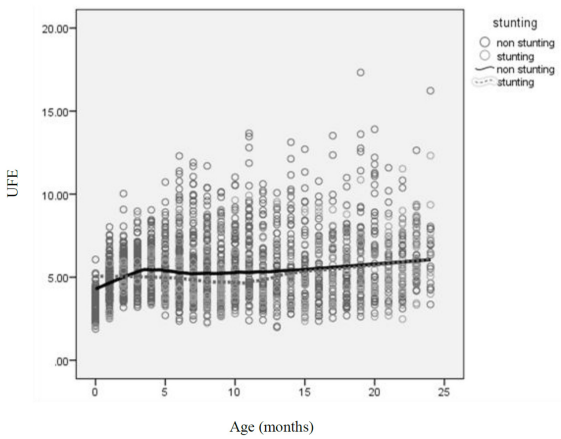

Figure 4. Scatter plot for UFE in stunted and non-stunted children according to age, showing the trend from birth to 24 months of age

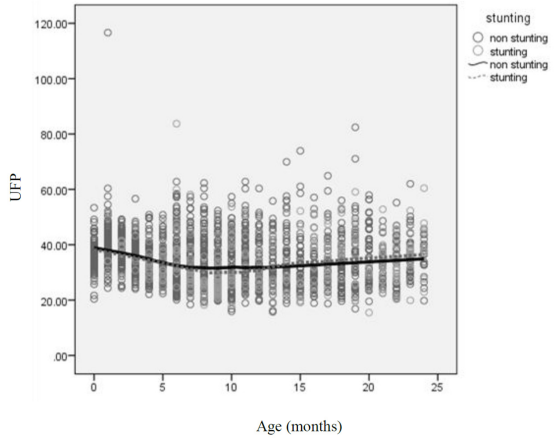

Figure 5. Scatter plot for UFP in stunted and non-stunted children according to age, showing the trend from birth to 24 months of age

Table 6. Mean UMA differences between stunted and non-stunted children according to age groups

Age groups	Stunted		Non-stunted		Mean difference, cm^2	95%CI	P value
	Mean UMA, cm^2	SD	Mean UMA, cm^2	SD			
0-6 mo	9.88	2.47	9.78	2.35	-0.10	-0.69 to 0.49	0.74
7-12 mo	11.31	2.25	11.79	2.12	0.48	0.07 to 0.91	0.02
13-24 mo	11.05	1.77	11.75	2.04	0.70	0.35 to 1.05	<0.001
Total	10.92	2.14	10.89	2.42	-0.03	-0.31 to 0.23	0.79

Table 7. Mean UME differences between stunted and non-stunted children according to age groups

Age groups	Stunted		Non-stunted		Mean difference, cm^2	95%CI	P value
	Mean UME, cm^2	SD	Mean UME, cm^2	SD			
0-6 mo	9.37	2.51	9.28	2.33	-0.09	-0.67 to 0.51	0.78
7-12 mo	10.90	2.28	11.30	2.19	0.4	-0.03 to 0.83	0.07
13-24 mo	10.50	1.83	11.18	2.12	0.68	0.32 to 1.05	<0.001
Total	10.43	2.19	10.37	2.44	-0.06	-0.31 to 0.19	0.06

The mean UMA and UME also appeared lower in stunted children aged 3-24 months compared to non-stunted children, as shown in the scatter plots (**Figures 6** and **7**).

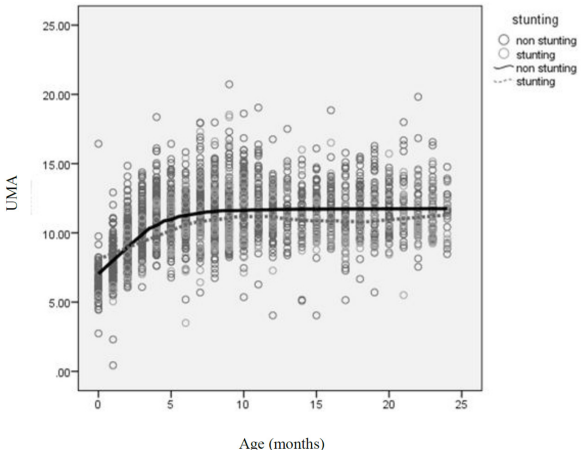

Figure 6. Scatter plot for UMA in stunted and non-stunted children according to age, showing the trend from birth to 24 months of age

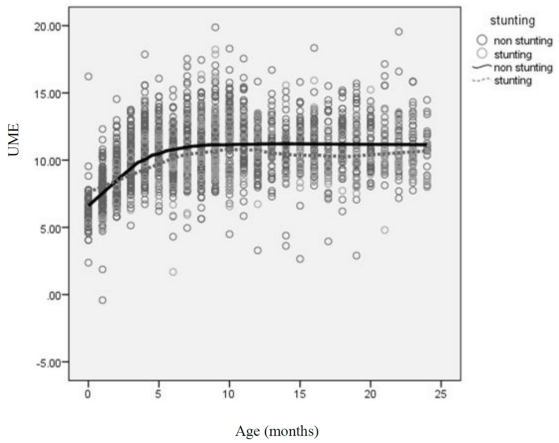

Figure 7. Scatter plot for UME in stunted and non-stunted children according to age, showing the trend from birth to 24 months of age

Discussion

In our study, stunted children aged 7-12 months had less upper arm fat (UFA, UFE, and UFP) than non-stunted children. In addition, upper arm muscle area, represented by UMA at 13-24 months and UME at 7-24 months of age, was significantly lower in stunted than non-stunted children. Therefore, stunted children less then 2 years of age had less fat and muscle area than non-stunted children, at certain ages.

Yemeni children aged 0-7 years indicated similar results, in that stunted children had significantly smaller UFA and UMA compared to non-stunted children at the same age.[13] There was also a significant correlation between upper arm muscle mass and height-for-age (HAZ) Z-scores in Kenyan children. The HAZ values were higher among children aged 9 months who had bigger upper arm muscle mass compared to those with smaller mass (-1.044 *vs.* -1.917, respectively; P <0.001) as well as at 24 months of age (–1.114 *vs.* –1.301, respectively; P=0.04).14 In Bolivia, stunted children aged 2-10 years had lower UMA Z-scores compared with non-stunted children (-0.43 *vs.* –1.23, respectively; P<0.001).[15]

Lipsberga *et al.* reported that among Latvian children aged 5-7 years, UFA and UMA correlated with body mass index (BMI) (in boys: r=0.35; P<0.05 and r=0.53; P<0.01, respectively; and girls: r=0.41; P<0.05 and r=0.48; P<0.01, respectively). However, correlation between fat and muscle mass with BMI should be interpreted cautiously, since taller children with normal fat may have lower BMI, in contrast to children with more muscular and bigger body frame having higher BMI.[16]

Slower fat and muscle growth may be explained by insufficient intake of energy and essential nutrients, and subsequent growth failure of long bones in stunted children. By 6 months of age, most children consume complementary food, therefore, food quality as well as quantity influences children's growth and development. Briend *et al.* suggested that energy deficient children without infection fulfill their energy requirement by fat mobilization. In this situation, the brain is prioritized, whereas other organs such as kidney, liver, thymus, and muscle receive lower energy supplies. Moreover, levels of hormones, such insulin and glucagon, as well as enzymes, are adjusted such that the organism enters an energy-sparing mode. When glucose is limited, brain and erythrocytes are supplied with energy from ketone bodies of fat tissues. However, glucose should always be available for brain and erythrocytes, Hence, the glucose should be supplied from other sources, i.e., glycerol breakdown from triglycerides, as well as amino acids alanine and glutamine breakdown in liver and kidney. The source of these two amino acids is muscle breakdown. Although the loss of amino acids from muscle in children with deficient energy and essential nutrients

but without infection is minimal, protein metabolism for muscle growth may be affected. The fat area of stunted children aged 7-12 months and muscle area at 13-24 months are smaller that those who are non-stunted, possibly indicating poor muscle growth after fat loss at younger ages.[17]

After 6 months of age, children often experience infection, especially diarrhea and respiratory tract infection. Children face a double burden of health problems due to limited energy and essential nutrient intake leading to anorexia, increased protein requirement for synthesis of an acute phase protein, glutathione, and to improve immune function. In the case of negative nitrogen balance, amino acids from muscle mass will be mobilized.[17]

The majority of muscle mass is located at the lower extremities, whereas muscle mass of the upper arm is considered to be representative of body muscle mass. Stunted children have diminished length of legs and arms, therefore, their muscle mass is also diminished. Growth faltering mainly occurs before 24 months of age, and length growth occurs at the lower part of the body. Before 6 months of age, children receive breast milk as the main source of nutrients, therefore, nutrient deficiencies and infection during this period of time is minimal. This phenomenon differs from the 7-24 month period, in which stunted children in our community probably received poor complementary food, leading to poor growth of muscle mass.[17]

Hormones may contribute to lower fat and muscle mass in stunted children, because fat regulates bone mass and linear growth. Fat and bone are considered to be endocrine organs that produce hormones to interact with the other organs, including the brain. Leptin is a hormone that affects bone density and catch-up growth. Wasted children may experience catch-up growth if their leptin levels are normal. However, this argument is still debated, since there was a case report of two children with congenital leptin deficiency with normal height. This phenomenon could be explained by multiple causes of stunting. A community report described a high prevalence of stunting among children with low prevalence of wasting. As we know, wasted children have smaller body fat mass. Another factor that causes stunting is dysentery. Moreover, stunting is probably accompanied by overweight, i.e., stunted-overweightness, and if stunted may stimulate linear growth, this fact could not be satisfactorily explained.[17]

Another essential nutrient for child growth and development is fatty acids. Fatty acids are an energy source and have a physiological role in cellular membrane structure, vision, skin integrity, wound healing, cardiac health, cognitive function, and the immune response. Essential fatty acids, such as linoleic acid or fatty acid n-6, and α-linolenic acid or fatty acid n-3, should be sufficiently provided in the food, because humans lack Δ-12 and Δ-15 desaturases, enzymes which synthetize essential fatty acids. Insufficient essential fatty acids in the diet affects growth, including fat and muscle mass. Linoleic acid-rich foods are sunflower seeds, nuts, soy oil, corn, and canola oil; whereas flaxseed, walnuts, and soy are rich in α-linolenic acid. Sources of essential fatty acids in animal foods are salmon, trout, egg, and poultry. Children with insufficient intake of fatty acids have higher risk of stunting with poor fat and muscle growth.[18] Tanzanian children aged 2-6 years with low intake of fatty acid n-6 had a high prevalence of stunting, however, increased intake of fatty acid n-9 correlated with stunting. Fatty acid n-9 is a non-essential fatty acid, which can be synthetized from fatty acid n-3 and n-6 using Δ-5 and Δ-6 desaturases, as well as elongases.[18]

Our study shows that smaller fat and muscle area in stunted children is clinically important. Decreased muscle mass is related to high mortality among children. Stunting and wasting indicate changes in body composition. Severely decreased fat and muscle mass diminishes the energy reserve that is imperative for vital organs, such as the kidneys, liver, heart, gut, and immune system. Previous studies have demonstrated a higher mortality among adults suffering from malnutrition with comorbidities of liver cirrhosis, cancer, or chronic lung disease. Children have smaller muscle mass compared to adults (23% in neonates *vs.* 43% in adults). But a child's brain is relatively larger for their body weight, therefore, children need more glucose. The glucose source for brain is muscle, therefore, reduced muscle mass effects higher mortality in children compared to adults.[17]

Stunted and wasted children with lower fat and muscle mass have a higher mortality risk. Therefore, health programs should prioritize children with combined stunting and wasting. Younger children

with infection are also important targets, as well as children born small-for-gestational age.[17]

A limitation of our study was the cross-sectional research design, as we were unable to identify changes in individual growth and nutritional status from birth to 24 months of age, nor could we describe factors causing differences in overweight and obesity prevalence, as well as differences in fat and muscle mass in stunted *vs.* non-stunted children. Also, in order to understand the role of leptin on fat and muscle mass in stunted and non-stunted children, the measurement of leptin levels is recommended. Leptin levels may be used as an indicator of whether extra calories given to stunted children will result in normal growth without any excess fat accumulation. A strength of our study was that the study population consisted of young children randomly selected from a whole province.

Based on the study results, we recommend a future longitudinal study from birth to 24 months of age to identify the change of overweight and obesity status, fat and muscle mass, and potential risk factors, especially with regards to food intake and infection. Leptin should be measured in order to precisely calculate the additional calories needed, in order to prevent obesity in stunted children. The differences in body composition among stunted and non-stunted children are indicative of their different nutritional requirements.

In conclusion, the prevalence of overweight or obesity among stunted children is significantly lower compared with non-stunted children. The highest prevalence of stunting is at 22 months of age in boys and 21 months of age in girls. Upper arm fat mass, as represented by UFA, UFE, and UFP, is lower among stunted than non-stunted children at 7-12 months of age. Upper arm muscle mass, as represented by UMA, is lower in stunted children aged 7-24 months, whereas UME is lower in stunted children aged 13-24 months.

Conflict of Interest

None declared.

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Original Article

The quantity and quality of anti-PRP induced by the new Indonesian DTwP-HB-Hib vaccine compared to the Hib vaccine given with the DTwP-HB vaccine

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Abstract

Background A phase II study of DTwP-HB-Hib vaccine compared to Hib (monovalent) vaccine given simultaneously with DTwP-HB vaccine has been done following the success of phase I study in infants, where the new DTwP-HB-Hib has excellent safety profiles and antibody responses in infants.

Objective To evaluate the titer (quantity), avidity, and bactericidal capacity (quality of anti-polyribosylribitol phosphate/anti-PRP), of a new combined Bio Farma DTwP-HB-Hib (pentavalent) vaccine, compared to the Hib monovalent vaccine given simultaneously with the DTwP-HB vaccine (DTwP-HB+Hib).

Methods The study was a prospective, randomized, open label, phase II trial. Subjects aged 6-11 weeks were allocated according to the randomization list. The pentavalent group received the DTwP-HB-Hib vaccine, while the monovalent group received the Hib monovalent and DTwP-HB vaccines separately. Immunizations were given in three doses with 28-day intervals. Blood specimens were taken before the first dose and 28 days after the last dose. We evaluated anti-PRP titers quantity (geometric mean antibody concentration/GMC) and seroprotection), followed by avidity and bactericidal (quality) testing. Titer and avidity of anti-PRP were tested using a modified version of the improved Phipps ELISA. Bactericidal capacity was evaluated using a Hib killing assay. Immune responses against other antigens in the vaccine were reported separately.

Results One hundred five subjects in the pentavalent group and 106 subjects in the Hib monovalent group were tested for anti-PRP titers. Only 102 specimens for each group were available for bactericidal testing, due to insufficient volume for testing. Both vaccines induced similar anti-PRP titers, for GMC and seroprotection. Avidity increases were 82.9% and 76.4% in the pentavalent and Hib monovalent groups, respectively. Bactericidal activities were 94.1% and 89.2%, respectively. Both avidity and bactericidal activity were not significantly different between groups.

Conclusion DTwP-HB-Hib vaccine induced anti-PRP quantity and quality comparable to those of the Hib monovalent vaccine given simultaneously with the DTwP-HB vaccine. **[Paediatr Indones. 2017;57:262-8; doi: http://dx.doi.org/10.14238/pi57.5.2017.262-8]**.

Keywords: *avidity; anti-PRP; bactericidal; DTwP-HB-Hib; immunization; titer*

Haemophilus influenza type b (Hib) causes infection with predominant manifestations of pneumonia, meningitis, and other invasive diseases. These infections occur primarily in children under 2 years of age, particularly in infants before Hib vaccinations became available.[1,2] In Asia, 23% of pneumonia cases are caused by Hib, while other etiologies include pneumococcus, staphylococcus, streptococcus, and viruses.[3] In Indonesia, pneumonia and meningitis cause an

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estimated 15.5% and 8.8%, respectively, of all deaths recorded in children under five years of age.[4,5]

The World Health Organization (WHO) has recommended worldwide incorporation of the Hib vaccination into all routine infant immunization programs, given after 6 weeks of age, preferably as a diphtheria-tetanus-pertussis (DTP)-based combination to allow for rapid integration into existing DTP vaccination schedules.[2] A phase I study comparing the Hib monovalent and the new DTwP-HB-Hib vaccines showed that both were immunogenic and well tolerated when administered as a single injection (Hib) in adults or as a primary dose (DTwP-HB-Hib) in infants, with 28-day intervals between doses.[6,7]

We aimed to evaluate the quantity of anti-PRP titer (GMC & seroprotection), avidity, and bactericidal capacity (quality of anti-PRP), of the new combined *Bio Farma* DTwP-HB-Hib (pentavalent) vaccine, compared to the currently approved Hib monovalent vaccine given simultaneously with the DTwP-HB vaccine (DTwP-HB+Hib).

Methods

The subjects were recruited for a prospective, randomized, open label, phase II study on the combined DTwP-HB-Hib vaccine. The study was conducted at 3 primary health centers in Bandung from July 2011 to January 2012. Subjects' parents provided written informed consent before enrollment. The study was conducted in accordance with the Helsinki Declaration and Good Clinical Practice Guidelines, with approval of the Ethics Review Committee of Universitas Padjadjaran Medical School.

The study population was comprised of healthy infants aged 6-11 weeks at the time of enrollment, of gestational age 37-42 weeks at delivery, with a birth weight of 2,500 – 4,000 g, and had received a single dose of monovalent hepatitis B vaccine at 0-7 days after birth, as proven by written vaccination documentation. At the time of enrollment, subjects were assigned to one of two vaccine groups by permuted-block randomization.

We administered the primary vaccinations to Indonesian infants according to the *Expanded Program on Immunization* (EPI) schedule, at 6, 10, and 14 weeks of age, after a birth dose of hepatitis B vaccine, as recommended by the WHO. Anti-PRP titers and avidity will be tested using ELISA, bactericidal activities will be tested using Hib bactericidal assay. The safety and immune response against other antigens in the vaccine were published separately.

The new liquid DTwP-HB-Hib (pentavalent) vaccine was produced by *Bio Farma*. This vaccine hasd5 antigens, with each 0.5 mL dose containing > 30 IU purified diphtheria toxoid, > 60 IU purified tetanus toxoid, > 4IU inactivated *Bordetella pertussis*, 10μg recombinant HBsAg, and 10μg Hib/PRP conjugated to tetanus toxoid. As a control, the DTwP-HB vaccine, also manufactured by *Bio Farma*, contains 4 antigens, with similar amounts, except for hepatitis B which had only 5 μg HBsAg per dose (0.5 mL). The Hib monovalent vaccine was imported (registered in Indonesia) and also contained 10μg Hib/PRP conjugated to tetanus toxoid per dose. Vaccines were administered at 6, 10, and 14 weeks of age, with 28-day intervals between doses. One group received the new DTwP-HB-Hib combination vaccine, while the other group received the DTwP-HB and Hib vaccines simultaneously. Vaccines were given intramuscularly in the external anterolateral region of the thigh.

Four-mL blood specimens were collected before the first vaccine dose and 28 days after the third dose to evaluate antibody responses. All assays were performed at the *Bio Farma Clinical Trial Laboratory*. The titer of antibodies to PRP were measured by the *Improved Phipps* enzyme-linked immunosorbent assay (ELISA), a competitive ELISA for measuring serum antibody levels to *Haemophilus influenzae type b*.[8,10]

Tests were done in duplicate, and a control serum was added to monitor variations between test plates. Anti-PRP antibody reference lot 1983 from the US *Federal Drug Association* (FDA) was used in all plates. Anti-PRP antibody concentration of >0.15 μg/mL was considered to be the minimum protection threshold titer and a concentration of >1.0μg/mL was regarded as the long-term protection threshold titer.

The percentage of protection (seroprotection) was calculated and differences between groups were evaluated using Chi-square or Fisher's tests. The GMCs with 95%CI were calculated by taking the log-transformation of individual concentrations and calculating the anti-log of the mean of these transformed values. Exploratory analyses were

performed to compare GMCs between the vaccine groups using Mann-Whitney test.

Avidity was tested using a modified ELISA, which included the use of isothiocyanate, a chaotropic agent.[8] After coating the plate with PRP antigen and incubating overnight, serum specimens were prediluted to a concentration of 0.5μg/mL anti-PRP antibodies. Ammonium thiocyanate at concentrations of 0.8, 0.4, 0.2, 0.1, and 0.05M were added to some wells of the plate, but not all, in order to later obtain an avidity index. The reactions were stopped when the well without ammonium thiocyanate reached an optical density of 1.0. Antibody avidity was expressed as the avidity index corresponding to the molar concentration of ammonium thiocyanate required to produce a 50% reduction in absorbance.[9] Differences in percentages of subjects with increased avidity between groups were evaluated using Chi-square or Fisher's tests.

The bactericidal assay was used to evaluate the capacity of anti-PRP antibodies present in the serum to bind and activate complement, leading to the killing of the bacteria. The Hib strain Hib-CB33 was cultivated, harvested, and diluted to a concentration of around 104 CFU/mL. Eleven, serial, two-fold dilutions of serum to be tested (starting at 1:4) were mixed with 3-4 week rabbit complement and 25 μg of bacteria. After 45 minutes of incubation, the number of surviving bacteria was determined by plating 5μg onto chocolate agar and counting the colonies after plate incubation for 18 hours. The serum bactericidal titer was defined as the inverse of the highest dilution that led to > 50% bacterial killing, and was compared to the negative control serum. The cut-off value was 4 BT (bactericidal titer).[9] The percentage of subjects with > 4 BT was calculated, and the difference between groups was evaluated using Chi-square test. Serum bactericidal antibody (SBA) geometric mean titers (GMT) were also calculated, and the differences between groups were evaluated using Mann-Whitney test.

Results

Of the 220 subjects recruited and randomly allocated to one of two vaccine regimens, only 211 subjects were available for immunogenicity analysis in phase I.[10] Likewise, in this study not all subjects were available for all assays. Anti-PRP titer was measured in 2012; avidity was evaluated in 2013; and the bactericidal assay was done in 2015, due to reagent availability. The number of subject samples available for each assay is shown in **Table 1**. Fewer specimens were available for the bactericidal assay due to lack of volume. The minimum required sample size was 100 per group, hence, the actual number of subjects' specimens fulfilled the minimum requirement.

Anti-PRP antibody measurements were based on GMC and percentage of infants with titers of >0.15 μg/mL and >1.0 μg/mL, are presented in **Table 2**. Before and after vaccination, there were no significant differences between the DTwP-HB-Hib and DTwP-HB+Hib groups, with regards to mean GMC, and percentage of subjects with anti-Haemophilus B conjugate (PRP-T) >0.15 μg/mL, and anti-PRP-T >1.0 μg/mL.

Table 1. Number of subjects' specimens available for each assay

Assays	DTwP-HB-Hib (n)	DTwP-HB+Hib (n)	Total (N)
Anti-PRP ELISA	105	106	211
Avidity ELISA	105	106	211
Bactericidal assay	102	102	204

The number and percentage of subjects with increased avidity is shown in **Table 3**. Both groups showed similar results, in terms of anti-PRP avidity.

As shown in **Table 4**, the DTwP-HB-Hib group had more subjects with bactericidal activity (titer > 4) than the DTwP-HB+Hib group, but the difference was not significant [94.1% *vs.* 89.2%, respectively; (P=0.205)]. The SBA GMTs were also not significantly different between groups, both before and after immunization.

Discussion

Table 2. Anti-PRP antibody concentrations by group, pre- and post-vaccination

Description	Pre-vaccination		P value	Post-vaccination		P-value
	DTwP-HB-Hib (n=105)	DTwP-HB +Hib (n=106)		DTwP-HB-Hib (n=105)	DTwP-HB+Hib (n=106)	
GMC, IU/mL (95%CI)	0.988 (0.925 to 1.055)	1.075 (0.9990 to 1.167)	0.989	12.612 (9.689 to 16.421)	11.663 (8.962 to 15.81)	0.876
Anti-PRP-T ≥ 0.15 μg/mL						
n	30	28		103	105	
%SP	28.6	26.4	0.726	98.1	99.1	0.621
(95%CI)	20.8 to 37.8	19.0 to 35.5		93.3 to 99.5	94.8 to 99.8	
Anti-PRP-T ≥ 1.0 μg/mL						
n	14	17		101	101	
%SP	13.3	16.0	0.579	96.2	95.3	1.00
(95%CI)	8.1 to 21.1	10.3 to 24.2		90.6 to 98.5	89.4 to 98.0	

DTwP-HB-Hib: diphtheria-tetanus-whole cell pertussis-hepatitis B-Haemophilus influenza type b vaccine; DTwP-HB+Hib: diphtheria-tetanus-whole cell pertussis-hepatitis B vaccine given simultaneously with Hib vaccine; n = number of available observations; % SP = seroprotection rate

Table 3. Anti-PRP antibody avidity by group

Avidity description	DTwP-HB-Hib (n=105)	DTwP-HB+Hib (n=106)	P value
Avidity increased, n(%)	87 (82.9)	81 (76.4)	0.245*
No avidity, n(%)	2 (1.9)	2 (1.9)	1.0**
Avidity constant, n(%)	9 (8.6)	14 (13.21)	0.325*
Avidity decreased, n(%)	7 (6.67)	9 (8.49)	0.617*

* Chi-square test; ** Fisher's exact test.

Table 4. Bacterisidal activity by group

Description	DTwP-HB-Hib (n=102)	DTwP-HB+Hib (n=102)	P value
Titer ≥ 4, n(%)	96 (94.1)	91 (89.2)	0.205*
GMT before immunization, BT (95%CI)	0	1.013 0.987 to 1.039	0.320**
GMT after immunization, BT (95%CI)	36.041 (27.321 to 47.534)	25.456 (18.471 to 5.075)	0.160**

* Chi-square test; ** Fisher's exact test.

The main objective of this study was to compare the quantity and quality of anti-PRP antibodies induced by the DTwP-HB-Hib pentavalent combination vaccine to the Hib monovalent vaccine given simultaneously with the DTwP-HB vaccine (DTwP-HB+Hib), as the primary vaccination in infants who had received a dose of hepatitis B vaccine at birth. After the primary series of vaccines was given, 98.1% of the DTwP-HB-Hib group and 99.1% of the DTwP-HB+Hib group had anti-PRP antibody titers above the conservative threshold of protection of 0.15 μg/mL. In addition, 96.2% of the DTwP-HB-Hib group and 95.3% of the DTwP-HB+Hib group had titers above 1.0 μg/mL. Our previous study showed that the immune response to Hib in the DTwP-HB-Hib pentavalent combination was not significantly different from the group that received separate administration of the monovalent Hib vaccine.[10]

Anti-PRP antibody avidities were similar in the pentavalent and Hib monovalent groups (P=0.245 to 1.0). Also, while the pentavalent group had a higher percentage of subjects (94.1%) with titer>4 (bactericidal activity), it was not significantly different from the monovalent Hib group (89.2%) (P=0.205). Nor were GMTs significantly different between groups,

both before and after immunization (P=0.320 and 0.160).

A 2009-2010 study in India in 661 infants aged 6 to 8 weeks using pentavalent combination vaccines with a 1-month interval between doses, reported anti-PRP titers of 100% seroprotection.[11] In addition, Hla *et al.* used a pentavalent vaccine given in 1-month intervals to 608 infants aged 6 weeks and showed similar results to ours: 100% short-term protection (anti-PRP > 0.15 μg/mL) and 95% long-term protection (anti-PRP > 1 μg/mL).[12] Another Indian study in 165 infants at 6, 10, and 14 weeks of age also found similar results to our study: at one month after the third vaccination, the percentages of infants who achieved the predefined protective antibody levels were 100% Hib short-term (≥ 0.15 μg/mL) and 95% Hib long-term (≥ 1.0 μg/mL) protection.[13] These three studies were conducted without control groups.

A Latin American study used pentavalent vaccines in 1,000 infants. Statistical comparisons following primary vaccination showed that, in terms of antibody response to the PRP antigen, the combined DTwP-HB/Hib vaccine was clinically non-inferior to the licensed DTwP-HB and Hib vaccines.[14] Furthermore, Rao *et al.* compared the new pentavalent vaccine to two groups: those who received the DTwP-HB+Hib vaccine (separate injections) and another registered pentavalent vaccine. They found that 98.32% of subjects in the new pentavalent vaccine trial group had seroprotective anti-PRP-T IgG antibody concentrations of ≥ 0.15 μg/mL, as compared to 100% and 98.94% of subjects in DTwP-HB+Hib and the other registered pentavalent vaccine groups, respectively.[15]

We found that the avidity increases were not significantly different between the pentavalent and Hib monovalent groups [82.86% *vs.* 77.14%, respectively (P=0.245)]. In contrast, a German study conducted in 90 infants with an immunization schedule at 3, 4, and 5 months using the DTaP-HB-Hib vaccine compared to separate injections given simultaneously showed significantly lower avidity in the combination vaccine group (P<0.0001).[9] A Myanmar study in 50 infants immunized at 6, 10, and 14 weeks of age compared several concentrations of Hib vaccines (10μg/mL and 2.5 μg/mL of a whole cell-based vaccine) showed avidity that was not significantly different (P=0.239).[8] In these two previous studies, anti-PRP avidity was not influenced by the whole cell-based pertussis vaccine, but, decreased by acellular pertussis base vaccine.[9] In our study both the pentavalent and Hib monovalent groups had subjects with avidity decreases of 6.67% and 8.49%, respectively (P=0.617). Some subjects also showed constant avidity before and after immunization, in both groups. Most subjects with non-increased avidity showed high titer concentrations of anti-PRP before immunization. A possible explanation for this phenomenon is the presence of maternal antibodies, which may prevent an immune response after immunization. The presence of maternal antibodies in the subjects before immunization may have also decreased the avidity after immunization, because this maternal antibody would eventually reduce significantly in time, especially after immunization at age 14 weeks or above.[16,17,18]

In our study, both groups also showed similar bactericidal activity (P=0.205). A study in Germany was conducted in 90 infants, with an immunization schedule of 3, 4, and 5 months using the DTaP-HB-Hib vaccine compared to separate injections given simultaneously. They also had not significantly different bactericidal activities.[9] The Myanmar study in 50 infants immunized at 6, 10, and 14 weeks of age comparing several concentrations of Hib vaccines (10 μg/mL and 2.5 μg/mL in a whole cell-based vaccine) also showed bactericidal activities which were not significantly different between groups.[9]

Some of our subjects had high anti-PRP and low SBA titers after immunization. Discrepancies can be expected because the SBA assay measures functional antibodies, regardless of isotype, to the whole organism, whereas anti-PRP IgG simply measures total IgG against capsular polysaccharide, regardless of antibody avidity. At the same time, infants with low anti-PRP IgG and high SBA titers were identified. There are several possible explanations for this observation. SBA titer may be the result of IgM and IgG, while we did not measure serum IgM alone. Another explanation for the discrepancy could be that the SBA assay measured functional activity against whole bacteria, and some infants may have naturally acquired functional antibodies against Hib subcapsular antigens. However, the ELISA measured only the concentration of anti-PRP (Hib polysaccharide capsule).[19]

Regarding the serum bactericidal antibody GMT, the pentavalent combination vaccine group reached 36.041 BT, while the separate injection group reached 25.456 BT (not significantly different, P=0.160). Townsend et al. found that SBA GMT in infants also reached 31 BT, similar to the SBA GMT of the trial vaccine.[19]

In conclusion, this study demonstrates that the new combined pentavalent DTP-HB-Hib vaccine induces comparable anti-PRP antibody titers, avidity, and bactericidal activity (anti-PRP quality) to the Hib monovalent vaccine given simultaneously with the DTwP-HB vaccine.

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Conflict of Interest

None declared.

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Original Article

Thrombospondin-1 and blood pressure in 7 to 8-year-old children born low birth weight and small for gestational age

Marlyn Malonda, Adrian Umboh, Stefanus Gunawan

Abstract

Background Thrombospondin-1 (TSP-1) is associated with endothelial damage, glomerular impairment, and hypertension. Low birth weight (LBW) and small for gestational age (SGA) children have higher risk of morbidity and mortality.

Objective To assess for a possible association between TSP-1 level and blood pressure in children who were born low birth weight and small for gestational age.

Methods We conducted a cross-sectional study from March to May 2015. Inclusion criteria were children who were born LBW and SGA in 2007-2008 at Prof. Dr. R. D. Kandou General Hospital, resided in Manado, North Sulawesi, had complete medical records, and whose parents consented to their participation. Exclusion criteria were children who were in puberty, obese, had renal disease, taking medications that affect blood pressure, or who were admitted to the hospital in the 2 weeks prior to enrollment. Data were analyzed using regression and simple correlation tests to assess for associations between TSP-1 and birth weight, as well as TSP-1 and blood pressure.

Results Subjects' mean TSP-1 level was 257.95 ng/dL. There was a strong negative correlation between TSP-1 and birth weight ($r=-0.784$; $P<0.0001$). In addition, there were strong positive correlations between TSP-1 level and systolic blood pressure ($r=0.718$; $P<0.0001$) as well as TSP-1 and diastolic blood pressure ($r=0.670$; $P<0.0001$).

Conclusion Higher TSP-1 is associated with higher systolic and diastolic blood pressure in 7 to 8-year-old children who were LBW and SGA at birth. Also, TSP-1 and birth weight have a strong negative correlation. **[Paediatr Indones. 2017;57:269-73; doi: http://dx.doi.org/10.14238/pi57.5.2017.269-73]**.

Keywords: *thrombospondin-1; blood pressure; low*

There has recently been an increasing interest in the influence of intrauterine life on the pathogenesis of chronic diseases. Infants who are born SGA have an increased risk of developing hypertension and cardiovascular diseases during adult life. Such conditions include dyslipidemia, glucose intolerance, hyperinsulinemia, and insulin resistance. *The Barker Hypothesis* postulated that these diseases are pre-programmed, due to an inadequate supply of nutrients during fetal development.[1-3] A history of SGA is a predictive factor of high blood pressure in early adulthood.[4-6] There have been many studies on the relationship between SGA and blood pressure, including other factors such as renal volume and function, plasma homocysteine, uric acid, the ACE gene, and P-selectin.[7] Building on the hypothesis, it is increasingly being recognized that an anti-angiogenic state is implicated in the

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pathophysiology of SGA pregnancies. The TSP-1, an inhibitor of angiogenesis, is a cellular matrix protein that was identified during cell injury. This TSP-1 is present in vascular circulation and binds to cellular receptors and other proteins in the vascular structure.[7]

Few studies have been done to determine a direct relationship between TSP-1 level, birth weight, and blood pressure in animals, especially in children born LBW and SGA.[8,9] Therefore, the objective of this study was to assess for possible associations between TSP-1 level and birth weight, as well as TSP-1 and blood pressure in 7 to 8-year-old children who were born LBW and SGA.

Methods

We conducted a cross-sectional study from March to May 2015 in Manado. The study was approved by the Ethics Committee of Sam Ratulangi University Medical School.

Subjects were healthy children who were born LBW and SGA seven to eight years prior to the study (2007-2008) in Prof. Dr. R.D. Kandou Hospital, had complete medical records, resided in Manado, North Sulawesi, and whose parents consented to participation. We excluded children who were in puberty, obese, had renal disease, taking medications that affect blood pressure, or who were admitted to the hospital in the two weeks prior to enrollment. Subjects underwent physical examination as well as anthropometric and blood pressure measurements. Body weight was measured using a platform beam balance scale. Blood pressure was measured in a quiet room in the subject's house, with the subject in a resting position , using a standard sphygmomanometer with an appropriately-sized cuff. Subjects underwent three readings, taken in five-minute intervals. The three readings were averaged to obtain a mean value.

The onset of the first Korotkoff phase was used to determine systolic blood pressure, and the onset of the fifth Korotkoff phase was used to determine diastolic blood pressure. Blood specimens were drawn with aseptic technique. Thrombospondin-1 levels were measured and analyzed using *Human TSP-1 by Quantikinine R&D Systems*.

The correlation between TSP-1 level and blood pressure was analyzed using regression and simple correlation tests. The minimum required sample size was calculated to attain 90% power, and P values < 0.05 were considered to indicate statistical significance. Statistical analysis was performed using *SPSS for Windows version 22.0*.

Results

During the study period, 128 LBW and SGA children were identified. However, 30 children's families could not be reached by phone, 26 had moved away, and 10 parents refused to participate. Hence, the eligible study population was 62 children, but 17 of these were excluded due to lack of cooperation (2), illness (5), and obesity (10). Therefore, 45 subjects were recruited into the study.

Table 1 shows the subjects' baseline characteristics. Subjects' mean birth weight are 2,000 was 1,980g and mean TSP-1 level was 257.95 ng/dL.

Table 1. Baseline characteristics of subjects

Characteristics	N=45
Gender, n(%)	
Male	18 (40)
Female	27 (60)
Age, n(%)	
7 years	29 (64.4)
8 years	16 (35.6)
Mean birth weight (SD), g	1,980 (148.24)
Mean TSP-1 (SD), ng/dL	257.95 (67.13)
Mean systolic BP (SD), mmHg	102.89 (9.91)
Mean diastolic BP (SD), mmHg	68.56 (8.02)

Figures 1, 2, and **3** show the relationships between TSP-1 levels and birth weight, as well as TSP-1 and systolic and diastolic blood pressures, respectively. There was a strong negative correlation between TSP-1 level and birth weight (r=-0.784; P<0.0001), a strong correlation between TSP-1 level and systolic blood pressure (r=0.718; P<0.0001), and a strong correlation between TSP-1 level and diastolic blood pressure (r=0.670; P<0.0001) in children with a history of LBW and SGA.

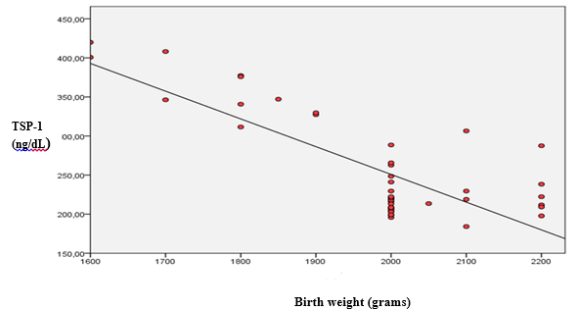

Figure 1. Thrombospondin-1 level (ng/dL) and birth weight (grams)

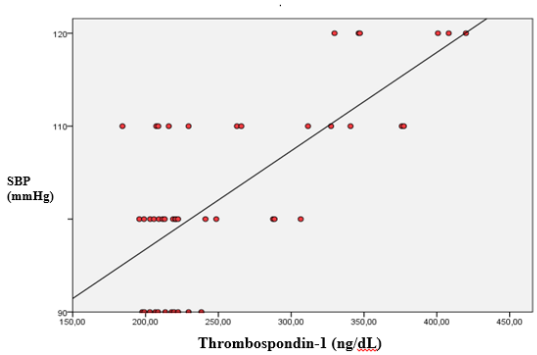

Figure 2. Thrombospondin-1 level and systolic blood pressure

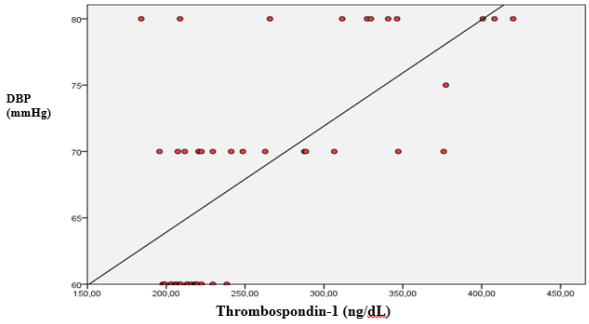

Figure 3. Thrombospondin-1 level and diastolic blood pressure

Discussion

To the best of our knowledge, this is the first study to investigate TSP-1 levels and blood pressure in children with a history of LBW and SGA. A previous study in an animal model determined that blood pressure increased due to several factors, including arterial tone, and increased TSP-1 expression. Our findings suggest that the higher TSP-1 levels correlate to higher systolic and diastolic blood pressures.

Strong positive correlations between TSP-1 level and systolic blood pressure ($r=0.718$; $P<0.0001$) and diastolic blood pressure ($r=0.670$; $P<0.0001$) were statistically significant. Our findings are consistent with those of Bauer *et al*.[8] They measured blood pressure with a transducer implanted in the tails of mice and found a positive correlation between TSP-1 level and blood pressure. Circulating TSP-1 inhibits endothelial nitric oxide synthase (eNOS) activation in endothelial cells. TSP-1-null mouse endothelial cells have inherently greater eNOS activity. Thus, TSP-1 limits production of the diffusible vasodilator nitric oxide (NO). Addition of intravenous TSP-1 to TSP-1-null mice stimulated vasoconstriction that can increase blood pressure. Higher TSP-1 circulating in blood correlated with increased blood pressure.[8]

Another study reported that in a rat model, TSP-1 level could increase diastolic blood pressure and mean arterial pressure (MAP). The integrity of the capillary wall was influenced by the level of TSP-1 circulating in blood. This result demonstrated for the first time that a matricellular protein acutely regulated blood pressure and cardiovascular responses to stress. Therefore, TSP-1 might act either as a direct vasoconstrictor or as an inhibitor of an endogenous vasodilator such as NO.[9]

Circulating TSP-1 is related to the risk of renal disease, especially hypertension in later life, along with other possible mediators that may disturb biomarker pathways (related to oxidative stress, endothelial dysfunction, angiogenesis, capillary rarefaction, and transforming growth factor β1). The TSP-1 enhances fibrosis and renal damage by its interaction with the biomarkers mentioned above . This TSP-1 is expressed in glomerulopathies and is considered an early marker of inflammation and fibrosis. Increased TSP-1 can inhibit NO, that in turn causes vasoconstriction, significantly increased glomerulosclerosis, glomerular matrix accumulation, podocyte injury, renal infiltration with inflammatory cells, and altered renal function parameters.[10-15]

Nevertheless, a comparison between human and animal models of TSP-1 levels and blood pressure should be viewed cautiously since TSP-1 levels and blood pressure in animal models may differ from human models.

We also found that TSP-1 levels in children with history of LBW and SGA were high, as well as

a negative correlation between TSP-1 level and birth weight ($r=-0.784$; $P<0.0001$). Similarly, previous publications showed that birth weight was a risk factor for increased TSP-1 levels.[16,17]

Isenberg *et al*. reported that the expression of TSP-1 in wild type mice born SGA and aged between 14 to 18 months demonstrated greater degrees of ischemia in the glomeruli and tubulointerstitium of the kidney and formed atherosclerosis in the vessel walls. TSP-1 level was related to birth weight. Also, TSP-1 increased in response to cellular injury. TSP-1 binding to CD47 receptors inhibits NO signaling by preventing cGMP synthesis and activation of its target cGMP-dependent protein kinase. The CD47 is a ubiquitously-expressed transmembrane protein that is present on all cardiovascular cell types and platelets. This potent antagonism of NO signaling allows TSP-1 to acutely constrict blood vessels, accelerate platelet aggregation, and if sustained, inhibit angiogenic responses.[16]

Andraweera et al. found that increased TSP-1 levels in SGA infants increase the risk of cardiovascular and renal disease in later life. They noted TSP-1 to be a prothrombotic and anti-angiogenic glycoprotein expressed in blood vessels. A single nucleotide polymorphism in the TSP-1 gene (TSP-1 2210A/G) was reported to be a significant risk factor for familial premature myocardial infarction, hypertension, and renal disease, which was associated with SGA, suggesting that TSP-1 polymorphism may be associated with the risk of vascular disorders across the course of life.[17]

In a rat study, TSP-1 expression was more prominent, especially in peritubular interstitial space. The percentage of glomeruli with positive intraglomerular TSP-1 staining was 19.3 (SD4.5) %; ($P<0.0001$). Tubulointerstitial TSP-1 expression in the cortex was 8.8 (SD 4.2) tubules/mm^2. TSP-1 has been shown to both inhibit endothelial cell proliferation and accelerate endothelial cell death (apoptosis). In addition, Kang et al. found that age-related TSP-1 expression was increased in both glomeruli and interstitium in rat kidneys. The increased TSP-1 expression strongly correlated with the degree of glomerulosclerosis, tubulointerstitial fibrosis, and glomerular hypertension.[18]

Accumulations of TSP-1 in vascular tissue may increase the blood pressure at a younger age, especially in children with a history of LBW and SGA, as related to intrauterine retardation. Even from the time of conception, TSP-1 may affect inhibition of vascular vasodilation and apoptosis of glomerular membrane.[6,7,9,15]

A limitation of this study was that the blood pressure measurements were performed on only one occasion. A 24-hour ambulatory measurement of blood pressure would be more accurate. Another limitation was that TSP-1 was measured only once, hence, further study is needed to monitor the TSP-1 levels in subjects.

In conclusion, 7 to 8-year-old children who were low birth weight and small for gestational age at birth have negatively correlated birth weight and TSP-1 level. Also, higher TSP-1 level is associated with higher blood pressure.

Acknowledgements

We were very grateful to Prof. Dr. Julius Lolombulan, MS, for his assistance with statistical analysis.

Conflict of Interest

None declared.

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Case Report

Preoperative intralesional injection of triamcinolone acetonide for a large head and neck lymphangioma in a baby: a case report

Julius July[1], Sophie Peeters[2]

Lymphangiomas (LMs) are uncommon congenital malformations of the lymphatic system, with an estimated incidence of one in 2,000 to 4,000 live births.[1] About half of these lesions are diagnosed at birth, and by two years of age, 90% of those with lesions have been diagnosed.[2] Histologically, LMs are benign lesions; however, they can pose a serious threat to the patient due to possible growth into surrounding structures, sometimes causing life-threatening complications. Treatment of large head and neck lymphangiomas in young infants is very challenging, due to the risk of surgical complications. Further challenges include the limited volume of blood loss that infants can tolerate, the lack of the option for radiotherapy or radiosurgery, and the high chance of life-threatening complications if the LM is not treated. Here, we report a case of a two-month-old baby girl presenting with a large head and neck lymphangioma. She was successfully treated with intralesional triamcinolone acetonide injections, followed by surgical resection of the lesion. **[Paediatr Indones. 2017;57:274-8; doi: http://dx.doi.org/10.14238/pi57.5.2017.274-8].**

Keywords: *head lymphangiom; triamcinolone acetonide; preoperative; intralesional injection*

The Case

The patient was a two-month-old girl presenting with a large head and neck lymphangioma on the left side (**Figure 1**). She was referred by a pediatrician for possible surgery and was rejected as a candidate for radiation therapy by a radiation oncologist. She presented with anemia and a hemoglobin level of 8.49 g/dL, likely due to the laceration on the posterolateral aspect of the mass that was actively oozing (**Figure 1**). The skin around the area was very thin and dark red. She had received multiple transfusions for anemia.

According to her mother, a dark red lump the size of a peanut was present behind the left ear at birth. The mass kept growing, despite multiple treatments. When she presented to our office, the mass was the size of an adult fist. The parents seemed quite frustrated and believed the LM to be untreatable at this point.

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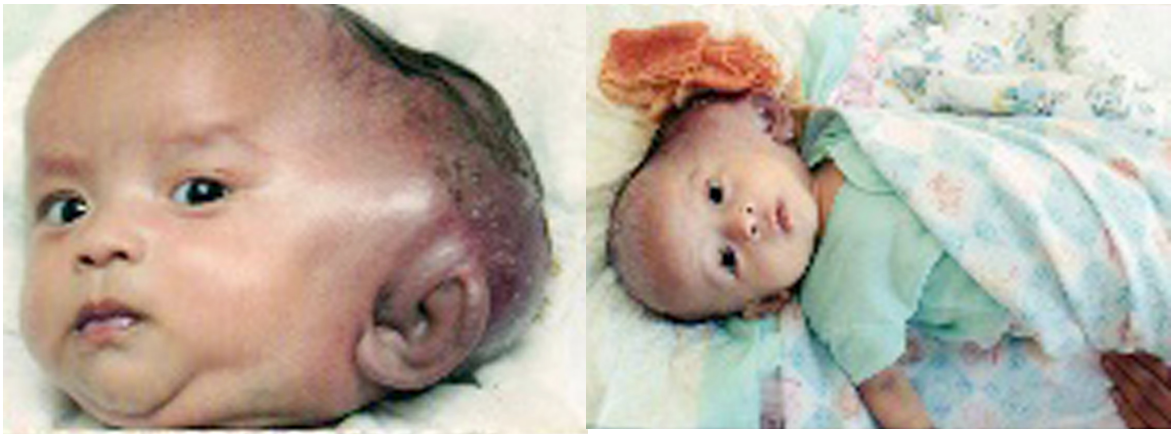

Figure 1. Two-month-old girl with a large left head and neck lymphangioma and a significant lateral inferior shift of her ear. The scalp vein was engorged and there was an oozing laceration on the posterolateral aspect of the mass.

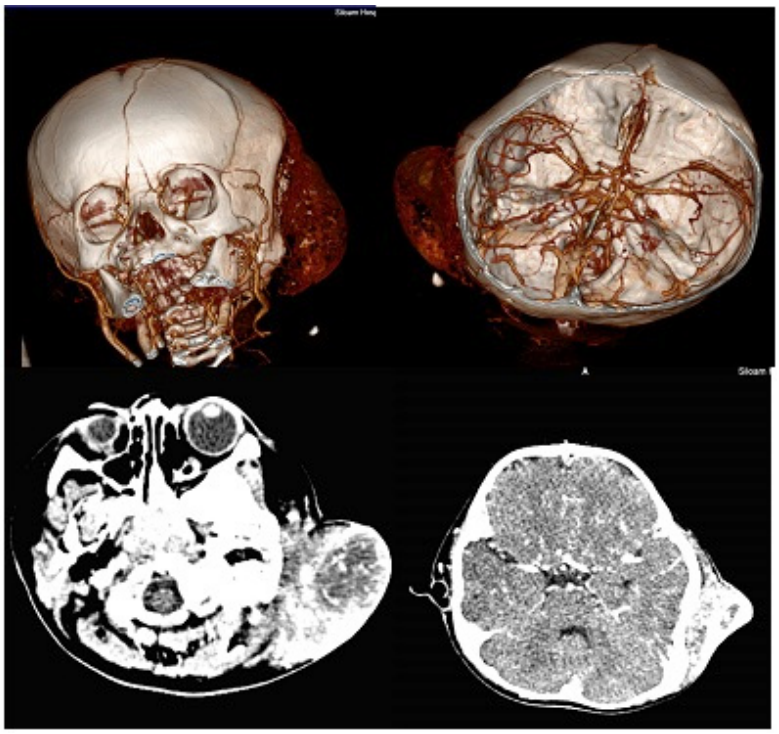

Figure 2. CT scan with contrast and 3D reconstruction.
Upper left: anterior view shows left retroauricular mass, 7.8 x 5.3 x 8.8 cm, highly vascularized and full of tortuous vessels. The scalp vessel was also congested, and the adjacent temporal bone had undergone remodelling. Upper right: axial view of 3D CT angiography showed no abnormal intracranial vessels. Note the remodelled temporal bone from an intracranial view. Lower left: axial cut of the contrast scan at the lower part of the lesion shows some calcifications and an intralesional hemorrhage. Lower right: the cut at the upper part of the lesion show no obvious pathology in the brain.

A CT scan showed a left retroauricular mass (**Figure 2**), measuring 7.8x5.3x8.8 cm, comprised of tortuous internal vessels, some calcifications, and an intralesional hemorrhage. The lesion was exerting a

mass effect on the temporal bone and eroding it. The intracranial anatomy was preserved. Both clinical and radiological findings were consistent with a hemangioma or lymphangioma.

We decided to give intralesional injections of 5mg/mL triamcinolone acetonide solution for a total dose of 7.5 mg at 3-week intervals, using a 25-gauge needle. The injections were performed at random,

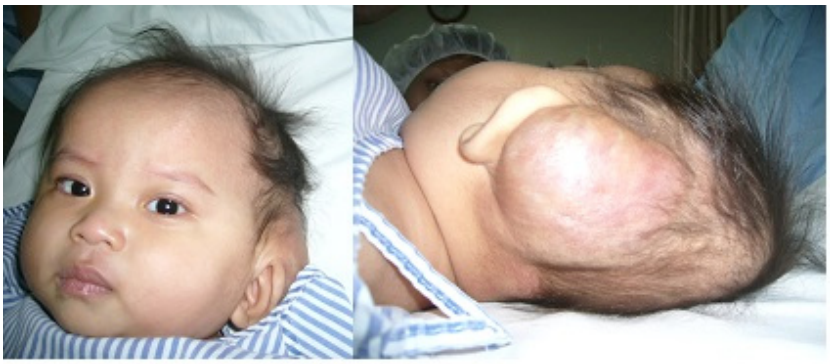

Figure 3. The lesion regressed significantly after ten sets of intralesional injections of triamcinolone acetonide. Left: Front view showing the position of left ear is almost normal. Right: side view, the mass behind the left ear regressed and the skin colour is almost normal.

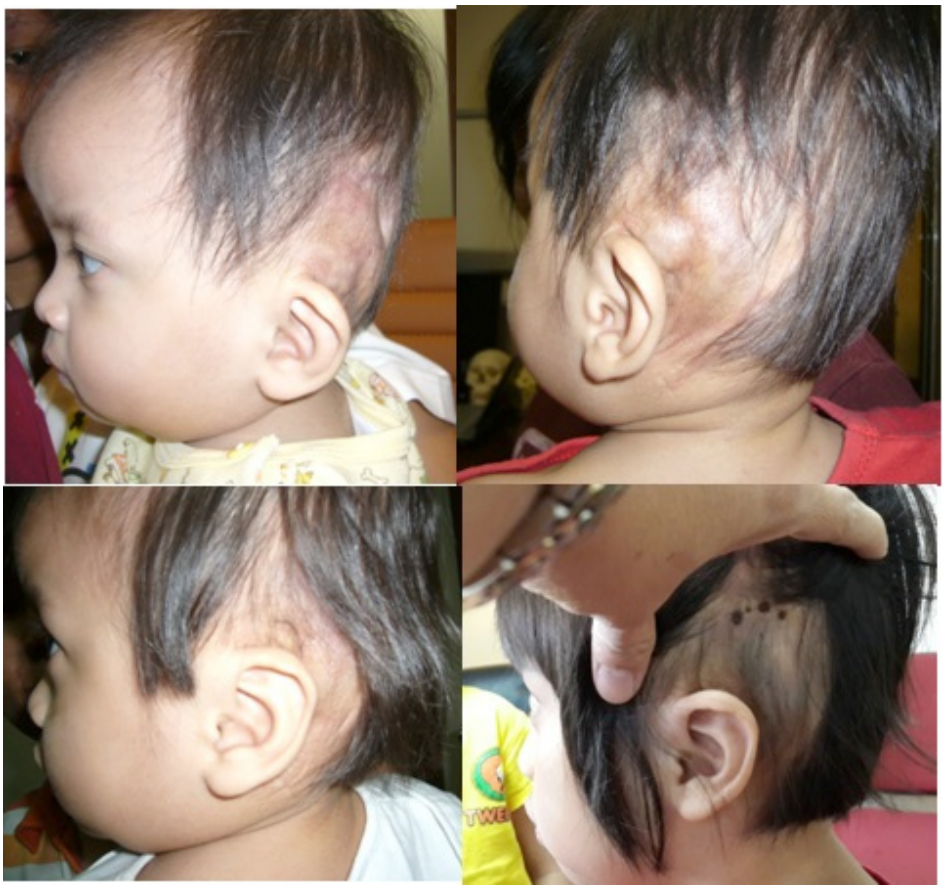

Figure 4. Upper left: 1 month after surgery. Upper right: 6 months after surgery. Lower left: 1 year after surgery Lower right: 3 years after surgery (age 4 years, height 92.5 cm).

at three to four different locations on the lesion per administration. The lesion regressed significantly after ten sets of injections (**Figure 3**).

Subsequently, we decided to proceed with surgical resection of the lesion. She recovered well after surgery, with no recurrence of the LM (**Figure 4**). The pathology report revealed the mass to be a lymphangioma.

Discussion

Lymphangiomas consist of a multitude of anastomosing lymphatic channels and encapsulated cystic spaces of different sizes, filled with proteinaceous or hemorrhagic fluid.[3] Lymphangiomas have been classified into four histological types: lymphangioma simplex, cavernous lymphangioma, cystic hygroma, and lymphangiosarcoma; potentially representing a continuum of pathological evolution.[2] They can be subdivided into micro- and macrocystic, corresponding to a cyst size less than or greater than 1 cm, respectively.[3] These lesions can be solitary or multifocal, with waxing and waning growth patterns.[3] The most common location of LMs is the head and neck region, but they can also occur on the trunk, extremities, face, or oral cavity.[2,3] Occasionally, the lesions can be painful and/or bleed; additionally, they have been associated with lymphopenia, leading to a higher risk of infections for these patients.[1]

The mortality rate of this pathology varies from 3.4 to 5.7%.[3] Lymphangiomas can be nicely defined on CT or MRI, and ultrasound should be carried out as well for a thorough work-up. In the past, surgery was the obvious treatment of choice with regard to lymphangiomas. Recently, an increasing number of studies have suggested more conservative, and possibly safer, management alternatives to surgery, but with mixed results. These include sclerotherapy, ultrasound-guided needle decompression of the cysts, or intralesional corticosteroid injections.[2,6]

Lymphangiomas are hypothesized to originate in locations corresponding to the six primary lymph sacs, secondary to closure of the embryological lymphatic tissue and of parts of those primitive sacs, which arise during the fifth week of gestation.[3,4] This particular case, and other cervicofacial LMs, most likely arose from the jugular sac.

As seen with our patient, these lesions tend to present as soft, doughy, poorly defined, and with intralesional hemorrhage. When the LM reaches a significant size, it may be subject to trauma, bleeding and infection. Though sometimes uninvolved, the overlying skin may demonstrate some lymphatic papules, or vascular cutaneous marks.[1] In addition, the skin color may have a blue shade to it, especially when the LM is macrocystic, or it may appear as a dark-red dome after intralesional hemorrhage. Internally, these masses are uni- or multi-loculated, with very thin walls. The cystic cavities contain clear, proteinaceous, serous fluid, which is highly concentrated in lymphocytes and macrophages.[1]

In fact, 45% of such stable lesions have shown spontaneous regression.1 If intervention is needed, the standard treatment for LMs used to be surgery, since a complete resection would almost guarantee elimination of any risk of recurrence. However, the main limitation with this approach was the potential damage to nearby vital structures.[2,3,5–7] Subtotal resection increased the already high recurrence rate (15-53%).[3,4,6] Intralesional injection of triamcinolone acetonide may offer a better option for presurgical treatment and improve the likelihood of complete resection of the lesion. The challenge for such a small baby is significant blood loss during surgery. Preoperative intralesional triamcinolon injections were very helpful in reducing the size of lesion in this infant, thus, minimizing surgical complications.

One case of tongue lymphangioma achieved a 90% regression and fewer bleeding episodes after intralesional steroid injections at 3-week intervals.[2] Similarly, an orbital lymphangioma regressed successfully with systemic corticosteroids.[8] The treatment time in the pediatric population should be very limited, since it may suppress adrenal function, thus leading to a risk of growth retardation.8 In our case, the child's growth rate was considered to be normal at the age of 4 years, with a height of 92.5 cm. The injections should be followed with surgical resection, once the lesion regresses to an operable size. Steroids are believed to inhibit the inflammatory response by diminishing the production of many cytokines, as well as of PDGF A and B, IL-6, TGF-beta 1 and 3, leading to a decrease in mass effect from the hypertrophy of the lymphoid tissue.[2,8] In addition, steroids may contribute to a decrease in spontaneous hemorrhages

by stabilizing the vasculature and inducing involution of vascular malformations.[8] Nonetheless, not every child responds to the treatment, and some cases have been reported in which the patient's LM either did not improve or progressed, despite intralesional steroid injections.[7]

Finally, other treatment options are electrocoagulation, cryosurgery, and laser surgery, if surgical treatment of lesions has a high risk of resulting in functional disability.

In conclusion, intralesional injections of triamcinolone acetonide may be an option for presurgical treatment. Short term use of preoperative intralesional triamcinolone injections was helpful for decreasing lesion size, thus reducing blood loss and improving the chances of complete resection of the lesion.

Conflict of Interest.

None declared.

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Original Article

Comparison of inflammation and oxidative stress levels by the severity of obesity in prepubertal children

Ni Luh Putu Surya Candra Eka Pertiwi, I Gusti Lanang Sidiartha

Abstract

Background Children with severe obesity have a much more adverse cardiometabolic risk factor profiles at a younger age. Inflammation and oxidative stress associated with childhood obesity may be important in the development of insulin resistance, atherosclerosis, and other comorbid conditions.

Objective To compare levels of high-sensitivity C-reactive protein (hsCRP) and malondialdehyde (MDA) by the severity of obesity in prepubertal children aged 6 to 10 years.

Methods We conducted a cross-sectional study at the Pediatric Nutrition and Metabolic Syndrome Clinic, Sanglah Hospital, Bali, from August to December 2015. Subjects were categorized into three body mass index (BMI) groups, according to the 2000 Centers for Disease Control and Prevention growth chart: overweight (85^{th}-94.9^{th} percentile), obese (95^{th}-98.9^{th} percentile), or severely obese ($\geq 99^{th}$ percentile). Plasma MDA and serum hsCRP were analyzed in blood specimens obtained at enrollment. Data were analyzed by Kruskal-Wallis test, followed by Mann-Whitney U test for post-hoc comparison between groups.

Results Subjects were 20 overweight children, 29 obese children, and 28 severely obese children. Levels of MDA were significantly higher in the severely obese [median 0.25 (IQR 0.1) μmol/L] than in obese subjects [median 0.19 (IQR 0.1) μmol/L; P=0.001], and than in overweight subjects [median 0.16 (IQR 0.1) μmol/L; P<0.0001]. Also, the severely obese children had significantly higher hsCRP levels compared to obese [median 3.2 (IQR 2.0) mg/L *vs.* 1.3 (IQR 1.6) mg/L, respectively; P<0.0001] and compared to overweight children [median 0.7 (IQR 0.6) mg/L; P<0.0001].

Conclusion Prepubertal children at the $\geq 99^{th}$ percentile for BMI (severely obese) are more likely to have significantly higher hsCRP and MDA compared to those in the obese and overweight groups. **[Paediatr Indones. 2017;57:279-84 ; doi: http://dx.doi.org/10.14238/pi57.6.2017.279-84]**.

Keywords: *obese; children; MDA; hsCRP*

Childhood obesity is increasing at an alarming rate throughout the world. The prevalence of obesity in children rose worldwide by 47.1% between 1980 and 2013.[1] If current trends continue, the number of overweight or obese children globally will increase to 60 million by 2020.[2] Although once considered a problem only of developed countries, the prevalence of obesity has also risen during the past 30 years in low and middle-income countries. At the same time, low and middle-income countries are still dealing with the prevalent public health issue of undernutrition, a situation often described as the "double burden of malnutrition." According to a recent report, 12% of children in Indonesia suffer from wasting, while a further 12% are

This study was presented at the P*ekan Ilmiah Tahunan Ilmu Kesehatan Anak VIII* (The 8th Child Health Annual Scientific Meeting), Makassar, South Sulawesi, Indonesia, September, 19-21, 2016.

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overweight. The economic cost of noncommunicable diseases, many of which is diet-related, was estimated to be $248 billion USD per year.[3]

Other data further suggested that compared to overweight and obese children and adolescents, youth with severe obesity have a much more adverse cardiometabolic risk factor profiles and are more likely to develop diabetes and cardiovascular diseases at a younger age.[4,5] Inflammation and oxidative stress associated with childhood obesity appears to be central to the development of insulin resistance and atherosclerosis, and may be important in the pathogenesis of other comorbid conditions.[6] A previous study documented that obese children with upper quartiles of C-reactive protein (CRP) and oxidative stress markers were more likely to have metabolic syndrome and abnormal lipid profiles, suggesting that inflammation and oxidative stress are interrelated, and might be associated with harmful health effects.[7]

To the best of our knowledge, no previous study has evaluated the levels of inflammatory and oxidative stress markers among overweight, obese, and severely obese prepubescent children. Therefore, the aim of this study was to compare high-sensitivity C-reactive protein (hsCRP) and malondialdehyde (MDA) levels by the severity of obesity in prepubertal children aged 6 to 10 years. We hypothesized that at a very young age, severely obese children have higher hsCRP and MDA levels compared to obese and overweight children.

Methods

We perfomed a cross-sectional study at the Pediatric Nutrition and Metabolic Syndrome Clinic, Sanglah Hospital, Bali, from August to December 2015. The eligibility criteria for participation in the study were children aged 6-10 years, with BMI at or above the 85th percentile for age and sex on the BMI growth chart from the Centers for Disease Control and Prevention (CDC),[8] and at prepubertal Tanner stage 1. Children with physical disability, history of acute infection, chronic diseases, chronic medication use, special diet, or whose parents refused to sign the informed consent were excluded from the study. We categorized subjects into three BMI groups of increasing severity: overweight (85th-94.9th percentile), obese (95th-98.9th percentile), or severely obese (≥ 99th percentile). The required sample size was determined by a formula of mean difference of two independent groups, with α of 0.05 and power of 80%. Therefore, a minimum of 20 patients per study group was required (total of 60 patients). Subjects were selected by consecutive, nonrandom sampling.

All anthropometric measurements and pubertal developmental staging (Tanner) were made by the same trained general physician and under the supervision of the same pediatrician, using standard protocols. Subjects' weights and heights were measured using a dual-purpose medical weight scale with height stadiometer. The stadiometer was checked for accuracy and the weighing scale was calibrated before the examinations. Height was measured without footwear and recorded to the nearest 0.1 cm from the highest point on the top of the subject's head. Body weight was measured and recorded to the nearest 0.1 kg, again without footwear and wearing lightweight clothing. We calculated the BMI percentile ranks using the CDC Child and Teen BMI Calculator.[9] Waist circumference (WC) was measured and recorded to the nearest 0.1 cm with a non-elastic tape at a point midway between the lower border of the rib cage and the iliac crest, at the end of normal expiration.

Patients were subjected to blood sampling for evaluation of MDA and hsCRP. The concentration of plasma MDA was measured using the *Bioxytech*® MDA-586 kit (*Oxis International*™). Serum hsCRP was measured by immunoturbidimetric method (analytic range 0.15-20 mg/L).[10] This study protocol was approved by the Research Ethics Committee of the Udayana University Medical School. Subjects' parents provided written informed consent.

Kruskal-Wallis test, followed by a Mann-Whitney U test for post-hoc comparison, was used to analyze the differences between groups. Data are presented as the mean, standard deviation (SD), median, and interquartile range (IQR). Results with $P<0.05$ were considered to be statistically significant for all data analyses. Statistical analyses were perfomed with *SPSS® version 22.0* software for *Windows®*.

Results

A total of 113 children were screened for inclusion

into this study. Thirty-six children were excluded (34 due to parental refusal and 2 due to acute infection). Therefore, 77 subjects were included in the final analysis (**Figure 1**).

Twenty overweight children (11 boys, 9 girls) with median age 9.5 (IQR 2) years and mean BMI of 21.1 (SD 1.8) kg/m^2 were compared to 29 obese children (14 boys, 15 girls) with median age 9 (IQR 2) years and mean BMI of 24.3 (SD 2.9) kg/m^2 as well as to 28 severely obese children (15 boys, 13 girls) with median age 9 (IQR 2) years and mean BMI of 28.2 (SD 2.4) kg/m^2 (**Table 1**).

Kruskal-Wallis test, followed by post-hoc Mann-Whitney U test, was used to evaluate differences in levels of MDA and hsCRP across the BMI groups. Kruskal-Wallis test revealed that significant differences existed in total MDA ($P<0.0001$) and hsCRP levels ($P<0.0001$) among the three BMI groups. **Figure 2** presents a box plot of MDA values for the three groups of children. Levels of MDA were significantly higher in severely obese [median 0.25 (IQR 0.1) μmol/L] than in obese subjects [median 0.19 (IQR 0.1) μmol/L; $P=0.001$), or in overweight subjects [median 0.16 (IQR 0.1) μmol/L; $P<0.0001$].

The serum concentration of hsCRP was also increased with the severity of obesity. The severely obese children had significantly higher hsCRP levels as compared to obese [median 3.2 (IQR 2.0) mg/L

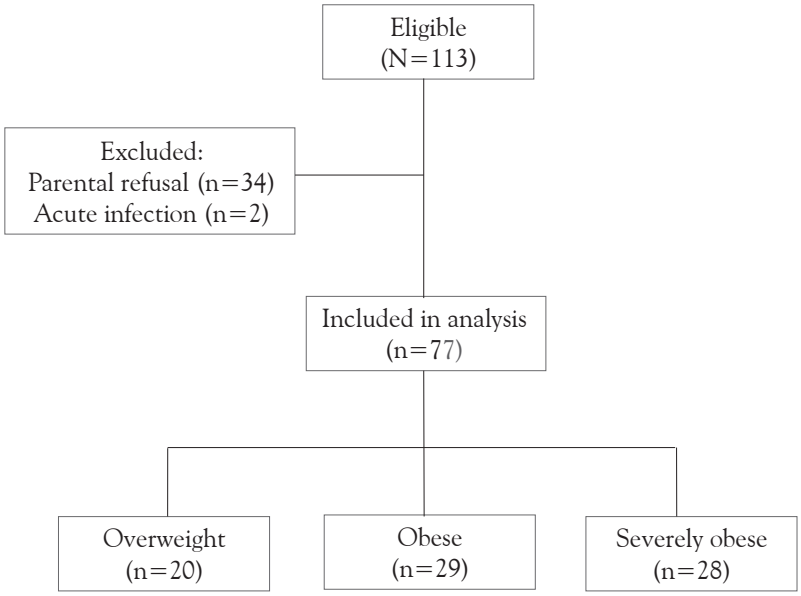

Figure 1. Study enrollment distribution

Table 1. Subjects' characteristics according to BMI category

Characteristics	Overweight (n=20)	Obese (n=29)	Severely obese (n=28)
Median age (IQR), months	9.5 (2)	9 (2)	9 (2)
Gender			
Male, n (%)	11 (55)	14 (48.3)	15 (53.6)
Female, n (%)	9 (45)	15 (51.7)	13 (46.4)
Mean BMI (SD), kg/m^2	21.1 (1.8)	24.3 (2.9)	28.2 (2.4)
Median weight (IQR), kg	38 (11.8)	41.0 (16)	51.5 (13.8)
Mean height (SD), cm	139.2 (10.2)	133.8 (8.9)	137.9 (9.3)
Mean waist circumference (SD), cm	75.7 (6.9)	76.1 (8.3)	82.4 (6.2)

SD: standard deviation; IQR: interquartile range

vs. 1.3 (IQR 1.6) mg/L, respectively; P<0.0001] and compared to overweight children [median 0.7 (IQR 0.6) mg/L; P<0.0001] (**Figure 3**).

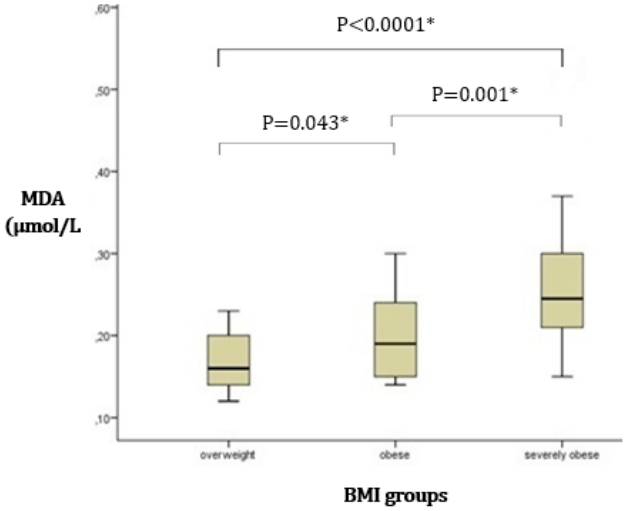

Figure 2. Comparison of MDA level based on BMI groups
*post-hoc analysis (Mann-Whitney U test)

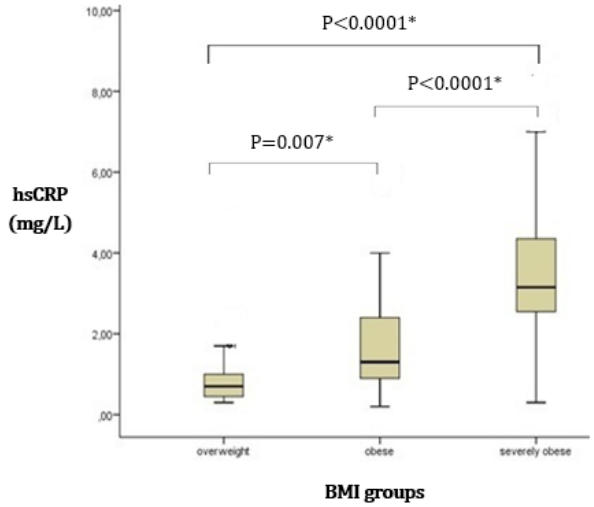

Figure 3. Comparison of hsCRP level based on BMI groups
*post hoc analysis (Mann-Whitney U test)

Discussion

Our study provides further confirmation to the close links between obesity, enhanced oxidative stress, and inflammation in prepubescent children. Firstly, levels of MDA as a biomarker for oxidative stress was increased more than 1.5-fold in severely obese compared to overweight children. Secondly, severely obese children had more than a 4-fold increase in hsCRP levels as a proinflammatory marker compared to the overweight group. Some previous studies among youths noted that subjects with more body fat had higher concentrations of inflammatory and oxidative stress markers. In a population-based study, Kelishadi *et al.* reported a significant correlation between CRP and oxidative stress markers (MDA and conjugated diene) in 512 children and adolescents aged 10-18 years with abdominal obesity.[7] Another study by Oliver *et al.* demonstrated simultaneous elevations in inflammatory and oxidative status in overweight and obese peripubertal children, when compared to healthy controls matched for age, gender, and fitness level. Obese children displayed significantly increased inflammatory cytokine (interleukin-6/IL-6) and systemic levels of lipid peroxidation (F2-isoprostanes). In addition, obese children displayed widespread alterations in their lipid profile, plasma glucose, and insulin compared to the control group.[11] Habib *et al.* conducted a case-control study and compared obese children and adolescents aged 5-17 years with healthy control participants matched for age and gender. They also showed significant elevations in proinflammatory adipocytokines (tumour necrosis factor-α and IL-6) and an oxidative stress biomarker (MDA), as well as significant decreases in antioxidant defense mechanisms (glutathione, zinc levels, and superoxide dismutase activity) among obese individuals compared to the control group.[12] Vehapoglu *et al.* recently found that at an early age (2-11 years), obese prepubescent children had concurrently elevated inflammatory (CRP) and decreased antioxidant status compared to underweight and normal-weight controls matched for gender. Furthermore, they observed significantly higher levels of fasting glucose, insulin, total cholesterol, triglycerides, homeostasis model assessment of insulin resistance (HOMA-IR), and homeostasis model assessment of β-cell function (HOMA-β), in obese prepubescent children compared to underweight and

normal weight children.[13]

Our study applied the 99th percentile cut-off point based on the longitudinal cohort *Bogalusa Heart Study*, that found severely obese children had higher rates of developing severe obesity in adulthood and much more adverse cardiometabolic risk factor profiles. They demonstrated that of those with severe obesity (BMI ≥99th percentile) at a mean age of 12 years, 100% developed to be adults with BMI ≥ 30 kg/m^2; 88% developed to have BMI ≥ 35 kg/m^2, and 65% developed to have BMI ≥ 40 kg/m^2. They also compared 6 cardiovascular risk factors (triglycerides, low density lipoprotein, high density lipoprotein, fasting insulin, systolic blood pressure, and diastolic blood pressure) in children based on BMI percentile. The results showed that 39% of children > 95th percentile and 59% of children ≥ 99th percentile had ≥ 2 risk factors, respectively, which was significantly greater than children in the 85th-95th percentile.[14] Previous studies have shown that CRP has now emerged to be one of the most powerful predictors of inflammatory markers for the evidence of cardiovascular events in adults.[15,16] There are currently no guidelines associating CRP levels and cardiovascular risk in children, but adults with a CRP > 3 mg/L are considered to have a 1.5 to 2-fold increased risk of cardiovascular disease.[16] In our study, severely obese children had markedly elevated hsCRP values [median 3.2 (IQR 2) mg/L] compared to overweight and obese children. These results suggest that the severely obese children in our study population may be at high risk of future cardiovascular disease according to the adult cut-off point.

Although a chronic inflammatory response is an established fact in obesity, the molecular determinants that trigger this response and maintain it in a sustained state are still poorly understood. Reactive oxygen species (ROS) and free fatty acids have, however, been proposed as potential contributors to this process.[17] Indeed, conditions that lead to increased oxidative stress are also known for their ability to lead to inflammation, in large part through the activation of nuclear factor kappa B (NF-kB).[18] In turn, activated inflammatory cells release high levels of ROS that potentiate the inflammatory response. Thus, the relationship between oxidative stress and inflammation is more complex than was originally thought, and it is clear that inflammation and oxidative stress are mutually inclusive and most likely operate by creating a cycle that exacerbates them.[19]

Regarding study limitations, we should acknowledge that this study does not show the temporal ordering of the association between oxidative stress, systemic inflammation, and severity of obesity. Further longitudinal investigations are needed to confirm these associations.

In conclusion, prepubertal children at the ≥ 99th percentile for BMI in the severely obese category are more likely to have higher hsCRP and MDA. Our data support the concept that at an early age, obesity activates biochemical mechanisms that might be responsible for long-term complications, thereby considerably increasing, if left untreated, the number of years of expected severe morbidity in patients.

Conflict of Interest

None declared.

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Original Article

The relationship between infant iron status and risk of neurological impairment

Buntat, Nurhayati Masloman, Johnny Rompis

Abstract

Background Iron deficiency (ID) is a commonly found nutritional disorder and a persistent problem, especially in Indonesia. Iron deficiency during the critical period in childhood brain development is estimated to cause irreversible damage that hinders infant development.

Objective To determine the relationship between infant iron status and neurological development.

Methods We conducted a cross-sectional study at the Growth and Development Outpatient Clinic, Prof. Dr. R. D. Kandou Hospital, Manado, from March to May 2015. By consecutive sampling, we obtained 44 healthy infants aged 7 to 10 months who fulfilled the inclusion criteria. Infants with a history of perinatal complications, such as head trauma, hypoglycemia, respiratory distress syndrome, infection, or malaria were excluded Subjects' serum hemoglobin and ferritin were examined for iron status. Infants' risk of neurological impairment was assessed by the Bayley Infant Neurodevelopmental Screener (BINS). Results were analyzed by descriptive analysis for the characteristics and Spearman's rank correlation coefficient analysis for the relationship between iron status and neurological development.

Results From 14 infants with ID, 8 infants had a high risk of developmental impairment. Of the 30 non-ID subjects, 4 infants had a high risk of developmental impairment. Of the 30 non-ID infants, 16 infants had a low risk of impaired development, while 2 infants with ID had low risk of developmental impairment. Spearman's rho revealed that infant iron deficiency was significantly associated with high risk of neurological impairment. ($r=-0.547$; $P<0.0001$).

Conclusion Lower serum ferritin levels (iron deficiency) is significantly associated with greater risk of impaired neurological development in infants aged 7-10 months. **[Paediatr Indones. 2017;57:291-4 ; doi: http://dx.doi.org/10.14238/pi57.6.2017.291-4]**.

Keywords: *iron deficiency; infant neurological development; Bayley Infant Neurodevelopmental*

Nutrition has an important role for achieving optimal growth and development in childhood.[1] Iron deficiency is a common nutritional disorder and one of the main causes of nutritional anemia, especially in Indonesia.[2] Ringoringo reported that the incidence of iron deficiency (ID) in infants aged 0-12 months was 7.6%.[3] In ID cases, not only tissue delivery of oxygen is compromised, but proliferation, growth, differentiation, myelinogenesis, immune function, energy metabolism, absorption, and biotransformation are also affected, leading to abnormal growth and behaviour.[4] So far, there has been no consensus

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reported incidence of ID in Indonesian infants aged 0-6 months, because no gold standard cut-off point for ferritin levels are available for the determination of ID deficiency in such infants. Currently, in Manado, particularly in Prof. Dr. RD Kandou Hospital, there is limited data on the prevalence of ID and the relationship between iron status and neurological development in infants younger than 12 months.

The aim of this study was to assess for a potential relationship between infant iron status and impaired neurological development.

Methods

This cross-sectional study was performed from March to May 2015 and included all consecutive healthy infants aged 7 – 10 months at the Growth and Development Outpatient Clinic, Prof. Dr. R. D. Kandou Hospital, Manado. We estimated a required sample size of 32 subjects, based on $\alpha=0.05$ and power=80%. The inclusion criteria were infants who registered as patients at our hospital (either at the clinic or by birth), with clear childbirth medical records, good nutritional status, born full term with birth weight ≥ 2,500 grams, Apgar score ≥ eight at the 5th minute, and with parental consent. The exclusion criteria were history of perinatal complications, including head trauma, hypoglycemia, respiratory distress syndrome, infection, or malaria.

Iron deficiency was defined as serum ferritin < 12 μg/mL,[5] with or without anemia. Anemia was defined as hemoglobin level < 11 g/dL.[6] Infant neurological development status was assessed by BINS. This examination is a combination of neurological and developmental evaluations of four conceptual areas of ability, i.e., basic neurological function, receptive function, expressive function, and cognitive processes. Results of the BINS examination were categorized as high, moderate, or low risk.[7] History-taking and physical examinations were performed to assess for infection and inflammation, weight (kg), and length (cm). Based on Z-score, nutritional status was classified as well-nourished (weight-for-length index -2SD to 2SD). Venous blood specimens (5 mL) were drawn by laboratory staff and tested for serum hemoglobin (Hb) and ferritin levels. The Hb level was measured with cyanmethemoglobin assay and serum ferritin level was measured with immunochemiluminiscence (ICMA).

Descriptive data analysis was used for the subjects' characteristics and correlative data analysis with Spearman's rho was used to assess for a possible association between infant iron status and neurological development. This study was approved by the Ethics Committee of Sam Ratulangi University Medical School, Manado.

Results

Initially, 50 children were eligible for this study. However, six children were excluded due to parental refusal to have infants' blood drawn, leaving a total of 44 subjects.

The prevalence of ID in our subjects was 32%. These subjects comprised of eight males and six females. The highest prevalence was found in the 10-month age group (8/14) (**Table 1**).

Table 1. Baseline characteristics of subjects

Characteristics	Iron deficient (n=14)	Non-iron deficient (n=30)	Overall (N=44)
Age group, n			
7 months	2	4	
8 months	3	6	
9 months	1	11	
10 months	8	9	
Mean age (SD), days			270.39 (31.78)
Sex, n			
Male	8	14	
Female	6	16	
Mean hemoglobin (SD), g/dL			11.37 (1.11)
Mean ferritin (SD), µg/L			40.59 (36.24)

Of the 14 infants with ID, 8 infants had high risk and two infants had low risk of impaired neurlogical development. Of 30 non-iron deficient infants 4 infants had high risk and 16 infants had low risk (**Table 2**). The relationship between infant iron status and neurological development was analyzed by Spearman's rho, revealing a moderate significant inverse association ($r=-0.547$; $P<0.0001$).

Table 2. Distribution of infants based on iron status and neurological development

Iron status \ Neurological development	Low risk, n	Moderate risk, n	High risk, n	Total, N
Deficient	2	4	8	14
Non-deficient	16	10	4	30

Discussion

Iron deficiency usually occurs in the second year of life, due to decreased iron intake and rapid growth in the first year. Normal infants need to absorb approximately 0.8 mg/day of dietary iron (0.6 mg for growth and 0.2 mg to replace ongoing losses).[8] Though anemia is a common manifestation of iron deficiency, other effects of iron deficiency on various tissues, organs, and systems are usually under recognized. Impaired brain development, as well as cognitive, behavioral, and psychomotor impairment are the most worrisome manifestations of iron deficiency. Studies have demonstrated that some of these impairments occurring during periods of brain growth spurts (<2 years age) may be irreversible.[9]

The prevalence of ID in our subjects was 32%, similar to that of a cross-sectional study by Apriyanti *et al*. in children aged 6-59 months.[10] We noted that eight males and six females had ID. Gender differences in ID reportedly only affect adolescents, as females are at higher risk due to menstruation and rapid growth.[11] The highest prevalence was found in the 10-month age group (8/14), perhaps as a result of inadequate iron in the diet, being bottlefed with non-iron fortified formula, or maternal iron status.[12,13]

The relationship between infant iron status and neurological development was analyzed using Spearman's rho, with $r=-0.547$ with $P<0.0001$. This finding was statistically significant. The lower the serum ferritin levels (iron deficiency), the higher risk of impaired neurological development. Other studies have reported a similar relationship between iron deficiency and neurological development in infants.[14-17]

A limitation of our study was that we did not assess other factors that may have affected iron status and neurological development, such as upbringing, genetics, quality of food, and maternal iron status.

In conclusion, lower serum ferritin level (iron deficiency) is significantly associated with higher risk of impaired neurological development. Hence, we recommend iron supplementation and some brain stimulation for infants with moderate to high risk of impaired neurological development. However, further study should be performed including analyzing several factors that can affect iron status and neurological development, such as upbringing, genetics, quality of food, and maternal iron status.

Conflict of Interest

None declared.

Acknowledgments

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Original Article

Quality of life in children with congenital heart disease after cardiac surgery

Sindy Atmadja, Tina Christina Tobing, Rita Evalina, Sri Sofyani, Muhammad Ali

Abstract

Background Major achievements in congenital heart disease (CHD) treatment over the past 20 years have altered the course and prognosis of CHD. Improvement of quality of life (QoL) is now a major goal of CHD treatment.

Objective To assess the QoL in children after cardiac surgery for CHD.

Methods A cross-sectional study was performed in children aged 2 to 18 years. The case group had 20 children with a history of corrective heart surgery in the 12 months prior to the study. The control group had 20 healthy children, age-matched to the case group. The QoL of both groups was assessed by Pediatric Quality of Life Inventory (PedsQL) Generic Core Scales. The same post-operative children were also assessed with the PedsQL Cardiac Module. Data were analyzed using T-test with $P < 0.05$ as the level of significance.

Results This study recruited 40 subjects: 20 post-operative and 20 healthy children. PedsQL Generic Core Scales assessment showed significant differences between groups in the physical function parameter of QoL ($P<0.05$) in children aged 13-18 years, but there were no significant differences in the social, emotional, and school function parameters. In children aged 2-12 years, there were no significant differences in physical, social, emotional, or school parameters. The PedsQL Cardiac Module assessment revealed that 35% of post-operative children was at risk for physical appearance problems, 80% was at risk for anxiety problems, 40% was at risk for cognitive problems, and 80% was at risk for communication problems.

Conclusion Thirteen to 18-year-old children with non complex CHD have poorer physical function than healthy children. Post operative children are at risk for physical appearance, anxiety, cognitive, and communication problems. **[Paediatr Indones. 2017;57:285-90 ; doi: http://dx.doi.org/10.14238/pi57.6.2017.285-90]**.

Keywords: *quality of life; children; cardiac surgery; congenital heart disease*

Congenital heart disease (CHD) is still a devastating problem in many countries worldwide.[1,2] Despite the measures taken to treat these patients, they may experience educational, physical, social, cognitive, and emotional problems. Thus these children are at risk for having a poor quality of life (QoL).[3]

Cardiac surgery is one of the many modalities of CHD treatment. Major achievements in cardio-surgical treatment over the past 20 years have altered the course and prognosis of CHD. Both palliative and corrective surgery aims to improve QoL.[4-7] Several studies have shown that the QoL of children with CHD after surgical correction was poorer in comparison with healthy children.[3,8,9] A study in Poland assessed QoL

This study was presented at *Pertemuan Ilmiah Tahunan Ilmu Kesehatan Anak VII/PIT IKA VII* (The 7th Annual Scientific Meeting of Child Health), Surabaya, October 31 – November 4, 2015.

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in subjects 10 years after corrective cardiac surgery. The study showed that exercise capacity, physical activity, and QoL of young adults with a history of surgical treatment for CHD were worse than observed in healthy peers, and their health status did not fulfill the definition of complete recovery.[10] These results were different from other studies which showed that post-cardiac surgical children have similar QoL with healthy children.[11,12] To date, there have been few studies on QoL in children after cardiac surgery in Indonesia. Thus we aimed to assess QoL in children with CHD after cardiac surgery compared to age-matched, healthy peers in Haji Adam Malik General Hospital, Medan, North Sumatera, Indonesia.

Methods

A cross-sectional study was performed from April 2014 to April 2015 in the Pediatric Cardiology Outpatient Clinic, Haji Adam Malik General Hospital, Medan, North Sumatera, Indonesia. The case group comprised children with CHD, aged 2 to 18 years, with a history of corrective heart surgery in the 12 months prior to study. The control group comprised healthy children without physical, emotional, social, and educational problems, who were age-matched to the case group. The subjects were divided based on age: 2-4 years, 5-7 years, 8-12 years, and 13-18 years. Hemodynamically unstable children, children with developmental and mental problems, and children with other chronic diseases that can affect QoL were excluded. Data were collected on subjects' age, sex, CHD type, surgery type, and residual CHD after surgery. Quality of life was assessed using the *Pediatric Quality of Life Inventory* (PedsQL). *The Generic Core Scales Version 4.0 PedsQL* was used in post-operative and healthy children, while the PedsQL Cardiac Module was used to assess disease-specific QoL in post-operative children.[3]

Data are presented as absolute numbers, means, and standard deviations. Comparisons between groups were analyzed by T-test. Level of significance was defined as $P < 0.05$. For the analysis, we used the *SPPS version 18* statistical package software. This study was approved by the Ethics Committee of University of Sumatera Utara Medical School, Medan.

Results

Subjects' characteristics are described in **Table 1**. There were 20 post-operative children and 20 healthy children. CHD types were ventricular septal defect (VSD), atrial septal defect (ASD), patent ductus arteriosus (PDA), tetralogy of Fallot (ToF), and transposition of great arteries (TGA).

Table 1. Demographic data of subjects

Characteristics	Post-operative children (n=20)	Healthy children (n=20)
Age, n		
2-4 years	8	8
5-7 years	4	4
8-12 years	3	3
13-18 years	5	5
Sex, n		
Male	15	15
Female	5	5
CHD type		
VSD	10	
ASD	1	
PDA	5	
ToF	4	
TGA	-	
Mean post-op duration (SD),* years	1.6 (0.6)	

*time from surgery until QoL examination

The post-operative children's characteristics are shown in **Table 2**. The surgery types were VSD closure, ASD closure, PDA ligation, and total correction for ToF. Four children had residual shunt after surgery. Almost all surgeries were conducted with cardiopulmonary bypass (CPB), with the exception of PDA ligation.

The results of the QoL assessment in children with CHD after cardiac surgery and control group are shown in **Table 3**. There were no significant differences of QoL in post-operative and healthy children in the age groups of 2-4 years, 5-7 years, and 8-12 years. However, in the 13-18-year age group, both parent and child reported poorer physical function in post-operative children ($P < 0.05$).

Disease-specific QoL results from the PedsQL Cardiac Module are shown in **Tables 4** and **Table 5**. Response scale in PedsQL was 0 (never a problem), 1

Table 2. Post-operative children's characteristics

No	Age, years	Sex	Diagnosis	CPB	Surgery type	Post-op duration, months	Residual shunt
1	3.4	M	Large VSD	+	VSD closure	25	-
2	4	M	VSD + PDA	+	VSD closure + PDA ligation	12	+
3	3	M	ToF	+	Total correction	14	-
4	4	M	Moderat VSD	+	VSD closure	20	-
5	2.6	F	Large PDA	-	PDA ligation	22	-
6	4	F	Large PDA	-	PDA ligation	22	-
7	3	M	Large PDA	-	PDA ligation	14	-
8	3	M	Large VSD	+	VSD closure	22	-
9	6.5	M	Moderate VSD	+	VSD closure	22	-
10	6	M	Moderate VSD	+	VSD closure	12	-
11	7	M	ToF	+	Total correction	12	-
12	5.6	F	ToF	+	Total correction	32	-
13	11	F	Large PDA	-	PDA ligation	24	-
14	10	M	Large VSD	+	VSD closure	24	-
15	11	M	Large ASD	+	ASD closure	26	-
16	18	M	Large VSD	+	VSD closure	12	-
17	14	M	Large VSD	+	VSD closure	36	+
18	17	M	ToF	+	Total correction	20	-
19	14	F	Large PDA	-	PDA ligation	14	+
20	13	F	Large VSD	+	VSD closure	13	+

CPB=cardiopulmonary bypass
Post-op duration=time from surgery until QoL examination in months

(almost never a problem), 2 (sometimes a problem), 3 (often a problem), and 4 (almost always a problem). These scales was converted to 0-100; being 0=100, 1=75, 2=50, 3=25, 4=0. We concluded a child was at risk of having a problem if the scale response was < 75. Both parent and child report showed that some postoperative CHD children had risk for physical appearance, anxiety, cognitive, and communication problems.

Discussion

Children with CHD are at risk for poor QoL. Despite the measures taken to treat these patients, they may experience interrupted education, limited movement and activities, disturbed social relationships with their parents and/or the environment, as well as problems in adjustment, including physical, social, cognitive, and emotional difficulties.3 Major achievements in cardio-surgical treatment over the past 20 years have altered the course and prognosis of CHD. Both palliative and corrective surgery aims to improve QoL.[4,5]

Our study showed that post-operative children aged 13-18 years had poorer physical function than healthy children, while social, emotional, and school function showed no significant differences in this age group. It was probable that this difference was caused by residual shunt, late diagnosis and late surgery in 13-18 years old group (mean post operative duration was 19 months prior to study). There were no significant differences of QoL in the age groups of 2-4 years, 5-7 years, and 8-12 years. The physical function parameter of the PedsQL includes walking, running, doing exercise or sport activites, taking a bath, and doing chores at home. Social function is how the child gets along with other children. Emotional function includes feelings of fear, sadness, worry, anger, and trouble sleeping. School function is how the child studies, such as difficulty paying attention, keeping

Table 3. PedsQL assessment in both groups

PedsQL	Post-operative children		Healthy children		P value
	N	Mean (SD)	N	Mean (SD)	
2-4 years					
Parent report					
Physical	8	88.9(6.32)	8	93.5(4.40)	0.11
Emotional	8	83.7(5.82)	8	83.1(6.51)	0.84
Social	8	87.5(8.86)	8	87.5(4.62)	0.10
5-7 years					
Parent report					
Physical	4	85.4(7.35)	4	92.5(5.00)	0.16
Emotional	4	81.2(10.3)	4	81.2(10.3)	0.10
Social	4	88.7(13.1)	4	88.7(6.29)	0.10
School	4	78.7(4.78)	4	80.0(7.07)	0.78
Child report					
Physical	4	91.3(3.90)	4	93.7(4.78)	0.90
Emotional	4	89.6(3.59)	4	87.5(5.00)	0.73
Social	4	93.7(4.78)	4	91.2(2.50)	0.39
School	4	78.7(2.50)	4	75.0(7.07)	0.25
8-12 years					
Parent report					
Physical	3	93.7(5.42)	3	98.3(2.88)	0.26
Emotional	3	66.6(10.4)	3	69.8(10.4)	0.10
Social	3	85.0(13.2)	3	88.3(7.63)	0.72
School	3	85.0(5.00)	3	81.6(2.88)	0.10
Child report					
Physical	3	91.3(3.36)	3	93.3(5.77)	0.14
Emotional	3	88.3(2.88)	3	91.3(5.77)	0.49
Social	3	83.3(7.63)	3	86.6(2.88)	0.51
School	3	78.3(7.63)	3	85.0(5.00)	0.11
13-18 years					
Parent report					
Physical	5	89.3(5.22)	5	92.1(4.41)	0.03
Emotional	5	80.0(14.5)	5	78.0(11.51)	0.81
Social	5	76.0(14.3)	5	76.0(6.51)	0.10
School	5	80.0(9.35)	5	80.0(6.12)	0.10
Child report					
Physical	5	89.35(7.17)	5	95.0(3.53)	0.00
Emotional	5	86.00(8.21)	5	84.0(4.18)	0.69
Social	5	85.00(13.2)	5	89.0(7.41)	0.57
School	5	84.00(6.51)	5	86.0(8.21)	0.23

up with studies, and missing work or school because of illness.[13]

A study in Tuzla, which also used the PedsQL to assess QoL, showed that post-operative children aged 2-4 years, 5-7 years, and 8-12 years had poorer QoL than healthy children in the parameters of physical, emotional, social, and school function. But in children aged 13-18 years, there were no significant differences in QoL.[3] The difference may be due to the Tuzla study including all types of surgery (palliative and corrective) and complex CHD. In contrast, our study only assessed QoL after corrective heart surgery with no complex CHD.

Disease-specific QoL assessment using the PedsQL Cardiac Module showed that post-operative CHD children were at risk for physical appearance, anxiety, cognitive, and communication problems. Physical appearance tends to be a problem among children aged 8-12 years and 13-18 years and was associated with post-operative scars. Most children felt ashamed of their disease and post-operative scars. These children are also at risk for anxiety problems before going to the hospital, meeting the physician, or having a medical procedure. Some also found it difficult to communicate about their heart disease with other people.

Table 4. PedsQL cardiac module assessment based on parent report

No	Age, years	Heart	Therapy	Physical appearance	Anxiety	Cognitive	Communication
1	3.4	100	100	100	25	66	25
2	4	85.7	-	100	75	66.6	33.3
3	3	96	-	91.6	93.75	91.6	75
4	4	92.82	-	100	81.25	83.3	91.6
5	2.6	92.8	-	91.6	56.25	83	66
6	4	96	-	83	62.5	75	75
7	3	96.4	-	83	31.25	83	33
8	3	75	-	83	93.7	75	66.6
9	6.5	96.4	-	83.3	75	75	50
10	6	100	-	83	81.25	80	75
11	7	96.4	-	83	62.5	80	83
12	5.6	89.2	-	83.3	43.75	65	50
13	11	100	-	41.6	68.75	85	66.6
14	10	92.5	-	80	50	80	50
15	11	89.2	-	65	56.2	55	75
16	18	85.7	100	66	66.6	80	75
17	14	100	-	75	75	80	80
18	17	92.8	-	75	68.75	85	58.3
19	14	89	-	70	56.25	70	75
20	13	89.2	95	66.6	75	80	83
Mean (SD)		94.5 (8.6)	98.3 (2.9)	81.9 (11.7)	62.5 (20.9)	76.2 (8.1)	64.7 (18.5)

Table 5. PedsQL cardiac module assessment based on child report

No	Age, years	Heart	Therapy	Physical appearance	Anxiety	Cognitive	Communication
1	6.5	96.4	-	66	50	70	66.6
2	6	96.4	-	75	81.25	85	75
3	7	96.4	-	75	62.5	70	66.6
4	5.5	96.4	-	91.6	50	50	50
5	11	100	-	58.3	62.5	90	50
6	10	92.8	-	83.3	87.5	60	58.3
7	11	92.8	-	50	50	55	66.6
8	18	82.14	100	75	68	50	83
9	14	96.4	-	50	50	85	58.3
10	17	92.8	-	83.3	50	75	50
11	14	96.4	100	58	50	65	66
12	13	92.8		66.6	81.25	75	75
Mean (SD)		100 (28.9)	100 (38.9)	64.1 (34.1)	52.6 (27.5)	69.2 (13.6)	64.1 (34.6)

Some limitations of this study were the small sample size (only 20 postoperative children) and not assessing the time between diagnosis and surgery. In conclusion, post-cardiac surgery children aged 13-18 years have poorer physical function than healthy children. These children are at risk for physical appearance, cognitive, anxiety, and communication problems.

Conflict of Interest

None declared.

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Original Article

Relationship between age at menarche and exposure to sexual content in audio-visual media and other factors in Islamic junior high school girls

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Abstract

Background In recent decades, girls have experienced menarche at earlier ages, which may have negative effects on health. Exposure to audio-visual media and other factors may influence the age at menarche, although past studies have produced inconsistent results. Objective To assess for relationships between the age at menarche and audio-visual media exposure, socio-economic status, nutritional status, physical activity, and psychosocial dysfunction in adolescent girls.

Methods This cross-sectional study was conducted from August to October 2015 in students from two integrated Islamic junior high schools in Medan, North Sumatera. There were 216 students who met the inclusion criteria: aged 10-16 years and experienced menarche. They were asked to fill out questionnaires that had been previously validated, regarding their history of exposure to audio-visual media, physical activity, and psychosocial dysfunction. The data were analyzed by Chi-square and Fisher's exact tests in order to assess for relationships between audio-visual media exposure and other potential factors with the age at menarche.

Results Of 261 female students at the two schools, 216 had undergone menarche, with a mean age at menarche of 11.6 (SD 1.13) years. There was no significant relationship between age at menarche and audio-visual media exposure (P=0.68). Also, there were no significant relationships between factors such as socio-economic and psychosocial status with age at menarche (P=0.64 and P=0.28, respectively). However, there were significant relationships between earlier age at menarche and overweight/obese nutritional status (P=0.02) as well as low physical activity (P=0.01). Multivariate logistic regression analysis showed that low physical activity had the strongest influence on early menarche (RP=2.40; 95%CI 0.92 to 6.24).

Conclusion Age at menarche is not significantly associated with sexual content of audio-visual media exposure. However, there are significant relationships between earlier age at menarche and obese/overweight nutritional status as well as low physical activity. **[Paediatr Indones. 2017;57:323-8 ; doi: http://dx.doi.org/10.14238/pi57.6.2017.1323-8].**

Keywords: *audio-visual; age at*

Puberty is a transitional period from childhood to adulthood. At the time of puberty, the development of secondary sexual phenomena in females include the breast and pubic hair growth as well as menarche.[1] Menarche is the first menstrual cycle. Generally, menarche occurs within 2 years of breast development, at a mean age of 12.8 years and an age range of 10 to 16 years.[1] The Basic Health Survey (*Riskesdas*) in 2010 reported that the national mean age at menarche was 13-14 years.[2]

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The age of puberty in girls is earlier now than in the previous century. The age at menarche in developed countries in Europe and the Americas has decreased an average of 2 to 3 months per decade in the last 100 to 500 years.[3,4] Earlier age at menarche has also been seen in girls in developing countries.[5,6] The age at menarche has important health implications, as early menarche has been associated with increased risk of cardiovascular disease and breast cancer.[7] The age at menarche is influenced by many factors such as race, ethnicity, exposure to audio-visual media with sexual content, and other influences that include socio-economic and nutritional status, physical activity, and psychosocial dysfunction.[3] Global information content is readily accessible to children and adolescents, many of whom have adopted bad habits such as watching movies and accessing sexual content from television, or the internet via computers and cell phones. Exposure to audio-visual media that contains sexual content is known to accelerate menarche at adolescents' early age.[8]

One Indonesian study stated that there were significant relationships between audio-visual media exposure and environmental influences with age at menarche in female teenagers although the results are inconsistent.[8] In previous studies from various countries, subjects had different cultural backgrounds, thus results were varied. A study on the age at menarche has been limited in students from religious-based environments with more intensive hours of learning. Therefore, we aimed to assess for associations between age at menarche and exposure to sexual content in audio-visual media as well as environmental factors in adolescent girls from integrated Islamic junior high schools.

Methods

This analytical study with a cross-sectional design was conducted at Siti Hajar and Darul Ilmi Murni Integrated Islamic Junior High Schools, Medan, North Sumatera, from August to October 2015. The 216 subjects who fulfilled the inclusion criteria were 10- to 16-year-old girls who had already experienced menarche. The exclusion criteria were girls who used hormonal drugs, had a history of chronic disease, or had congenital disorders. The data about the exposure audio-visual media containing sexual content were obtained from questionnaires.

The staging system utilized most frequently was that published by Marshall and Tanner and the sequence of changes, commonly referred to as "Tanner stages", was described below (**Figure 1** and **Table 1**).

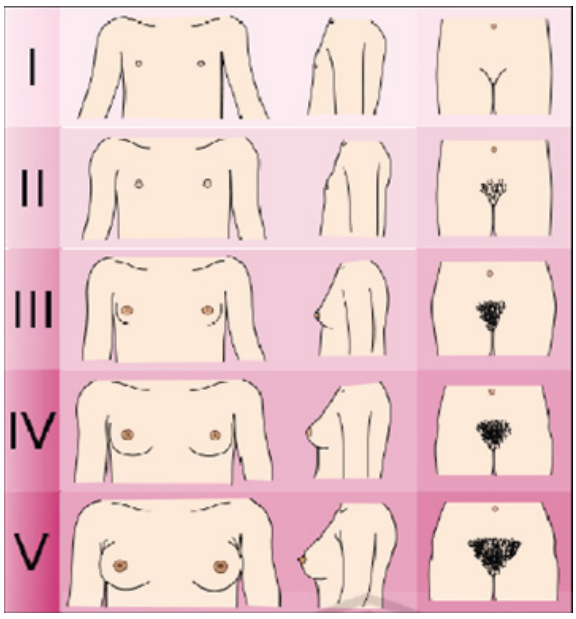

Figure 1. Female's Tanner Stage

Tabel 1. Female's Tanner Stage

Stage	Breast	Pubical hair
Stage 1	Prepubetal	Prepubertal (can see velus hair similar to abdominal wall)
Stage 2	Breast bud stage with elevation of breast and papilla; enlargement of areola	Sparse growth of long, slightly pigmented hair, straight or curled, at base of penis or along labia
Stage 3	Further enlargement of breast and areola; no separation of their contour	Darker, coarser and more curled hair, spreading sparsely over junction of pubes
Stage 4	Areola and papilla form a secondary mound above level of breast	Hair adult in type, but covering smaller area than in adult; no spread to medial surface of thighs
Stage 5	Mature stage: projection of papilla only, related torecession of areola	Adult in type and quantity, with horizontal distribution ("feminine")

Subjects' nutritional status was obtained by calculating actual body weight percentage (actual weight size) on ideal body weight, classified according to Waterlow 1972 as follows: obese > 120%, overweight >110-120%, normal 110-90%, malnutrition 70-90%, and severe malnutrition < 70%. Then grouped into two categories, namely groups with undernutrition-normal nutrition and groups with overweight-obese.

Physical activity from questionnaires about the respondents' daily activities within a 24-hour period divided to: light activity (when 75% light activity and 25% moderate and heavy activity), moderate activity (when 40% light activity and 60% moderate and heavy activity), and heavy activity (when 25% light activity and 75% moderate and heavy activity). Then grouped into two categories: group with inadequate activity (subjects with light activity) and adequate activity (subjects with moderate and heavy activity).

Socio-economic status by parental monthly income which devided into high and low socio economic. High socio economic status if the parent had monthly income more than standar minimum wage in city of Medan and low socio economic status if the parent had monthly income belowthe standar minimum wage in city of Medan which state by Governor of North Sumatra Rp. 2.037.000/month and psychosocial dysfunction was assessed by the *Pediatric Symptom Checklist 35 (PSC 35)* questionnaire. This study was approved by the Ethics Committee of the University of Sumatera Utara Medical School. Informed consent was obtained from subjects' parents.

The data were analyzed using SPSS, with Chi-square and Fisher's exact tests to assess for a possible relationship between age at menarche and audio-visual media exposure and other factors. Multivariate logistic regression analysis was used to analyze potential associations between age at menarche and audio-visual media exposure, psychosocial dysfunction, socio-economic status, nutritional status, and physical activity.

Results

Of 261 female students aged 10 to 16 years who underwent initial screening, 216 had experienced menarche and met the inclusion criteria. Subjects reported their age at menarche, then they were allocated to one of two groups: early menarche (at <10 years of age, 35 subjects) and normal menarche (at 10 to 16 years of age, 181 subjects).

Subjects' mean age at menarche was 11.64 (SD 1.13) years and their ethnicites consisted of 34.3% Javanese, 33.8% Batak, 27.3% Malay, and 4.6% others. Parental occupations also varied, but the majority were entrepreneurs (57.4%). All subjects were above Tanner stage 2 (**Table 2**).

Table 2. Characteristics of study subjects

Characteristics	N=216
Mean age at menarche (SD), years	11.64 (1.13)
Ethnicity, n (%)	
Javanese	74 (34.3)
Batak	73 (33.8)
Malay	59 (27.3)
Others	10 (4.6)
Parental occupation, n (%)	
Government employee	71 (32.9)
Entrepreneur	124 (57.4)
BUMN* employee	21 (9.7)
Tanner stage, n (%)	
Stage 1	0
Stage 2	0
Stage 3	115 (53.2)
Stage 4	89 (41.2)
Stage 5	12 (5.6)

**BUMN=Badan Usaha Milik Negara* (state-owned enterprise)

Fisher's exact test revealed no significant relationship between exposure to audio-visual media with sexual content and age at menarche (P=0.68). We noted that over 90% of girls in both groups had been exposed to audio-visual media with sexual content. We also evaluated several other factors that could potentially influence the age at menarche such as socio-economic status, nutritional status, physical activity, and psychosocial dysfunction. We found that the incomes of the majority of the subjects' parents were above the urban minimum wage (UMK) and their parents' occupations varied; most of them were entrepreneurs with high socio-economic status (**Table 3**). Fisher's exact test revealed no significant relationship between age at menarche and parental income (P=0.64). Chi-square test revealed a significant relationship between nutritional status and age at menarche (RP = 2.02; 95%CI 1.09-3.7; P=0.02). Significantly more overweight-obese

subjects underwent early menarche than normal menarche. We also found that early age at menarche was significantly associated with inadequate physical activity (RP=2.46; 95%CI 1.17 to 5.16; P=0.01). Subjects with inadequate physical activity had 2.46 times higher possibility of early menarche, compared to those with adequate physical activity. Chi-square test revealed no significant relationship between psychosocial dysfunction and age at menarche (P=0.28) (**Table 3**).

Logistic regression analysis was used to determine the factor with the strongest association with early menarche. Multivariate analysis showed this factor to be physical activity, with RP = 2.40, which indicated that subjects with inadequate physical activity had 2.40 times the risk of early menarche, compared to those with adequate physical activity but statistically was not significant (95%CI 0.92 to 6.24) (**Table 4**).

Discussion

In our study, subjects' mean age at menarche was 11.64 (SD 1.13) years, which was earlier than reported by the Ministry of Health (*Riskesdas*) in 2010 (13-14 years).[2] It appears that age at menarche in Indonesian children has gotten earlier in the last few years, consistent with a Yogyakarta study in 2013 which showed an early mean age at menarche of 11.8 years.[8]

Age at menarche may be influenced by many factors, one of which is exposure to audio-visual media with sexual content. Sexual stimuli from directly observing sexual activity causes the hypothalamus to stimulate secretion of specific hormones, which may eventually influence the process of reproductive organ maturity.[8] However, we found no significant

Table 3. Relationship between age at menarche with exposure to audio-visual media with sexual content, socio-economic status, nutritional status, physical activity, and psychosocial dysfunction

Characteristics	Menarche		RP	95%CI	P value
	Early (n=35)	Normal (n=181)			
History of exposure, n (%)					
Exposed	32 (91.4)	169 (93.4)			0.68**
Not exposed	3 (8.6)	12 (6.6)			
Socio-economic status, n(%)					
High	33 (94.3)	174 (96.1)			0.64**
Low	2 (5.7)	7 (3.9)			
Nutritional status, n (%)					
Overweight-obese	20 (57.1)	66 (36.5)	2.02	1.09 to 3.71	0.02*
Undernutrition-normal weight	15 (42.9)	115 (63.5)			
Physical activity, n (%)					
Inadequate	27 (77.1)	98 (54.1)	2.46	1.17 to 5.16	0.01*
Adequate	8 (22.9)	83 (45.9)			
Psychosocial dysfunction, n (%)					
No problem	22 (62.9)	96 (53)			0.28*
Problem	13 (37.1)	85 (47)			

* Chi-square test, ** Fisher's exact test

Table 4. Multivariate analysis of the potential factors influence the age of menarche

Variables	Coefficient	RP	95%CI	P value
Audio-visual media exposure	-0.16	0.86	0.21 to 3.44	0.83
Psychosocial dysfunction	-0.45	0.64	0.29 to 1.40	0.26
Parental income	-0.74	0.47	0.08 to 2.70	0.40
Nutritional status	0.56	1.75	0.76 to 4.02	0.19
Physical activity	0.87	2.40	0.92 to 6.24	0.07

relationship between age at menarche and exposure to audio-visual media with sexual content (P=0.68). Similarly, a Banten study reported no significant relationship between age at menarche and audio-visual media exposure (P=0.11).[9] However, an American study in 2005 indicated that there was a significant relationship between media with sexual content and girls' sexual maturity, and children who underwent early sexual maturity had sexual interest in seeing sexual content in movies, television and magazines.[10] In addition, a Yogyakarta study showed a significant relationship between age at menarche and audio-visual media exposure. Another factor that influenced sexual maturation in children was cultural background.[8] In our study, subjects were female students from integrated Islamic junior high schools which practiced an intensive learning system (full day school).

We also assessed other possible factors that could affect age at menarche including socio-economic status, nutritional status, physical activity, and psychosocial dysfunction. Good socio-economic status has been associated with early age at menarche in Indian girls, possibly due to parents' providing sufficient nutrition for their children.[11] A Nigerian study compared the age at menarche in girls from different socio-economic levels and found that those from middle and upper socio-economic classes underwent menarche earlier than those in the lower socio-economic class.[12] However, we found no significant relationship between age at menarche and socio-economic status (P=0.64). Almost all of our subjects were from good socio-economic background, as their family incomes were above the average minimum wage in Medan. We found no significant difference in age at menarche between the low and high socio-economic groups.

Nutritional status is considered to be an influencing factor in the development of puberty. Age at menarche and nutritional status have been correlated with body fat, with the hormone leptin playing an important role. Serum leptin concentration had a significant relationship with percentage of body fat. Leptin plays an important role not only in appetite for food, but also in the onset of puberty.[13] A Medan study conducted in 2012 showed that body mass index (BMI) was associated with menarche age.[14] Similarly, a French study showed that there was a significant relationship between good nutritional status/obesity and early menarche in female teenagers.[15] We also found in bivariate analysis a significant relationship between overweight/obese nutritional status and early menarche age (PR=2.02; 95%CI 1.09 to 3.71; P=0.02). The school administrations which this study was held are very concerned about nutritional needs of the students, as they provide lunch and snacks during recess, in order to fulfill students' needs for good nutrition, despite their hectic schedule.

Energy expenditure through physical activity is known to influence age at menarche. Intensive exercise tends to decrease gonadotropin and ovarian hormone production. Under conditions of excessive energy expenditure, the short-lived luteal phase, lower FSH levels, and higher prolactin levels can delay onset of puberty.[7] We, too, found a significant relationship between inadequate physical activity and early age at menarche (RP=2.46; 95%CI 1.17 to 5.16; P=0.01) in bivariate analysis. Similarly, a US study on adolescents who performed heavy physical activity prior to puberty had slower onset of puberty than adolescents with moderate physical activity. Low physical activity accelerates sexual maturation which causes early age at menarche.[16] In our study, most of the female students used personal vehicles to go to and from school.

Psychosocial dysfunction may result from stress-induced emotional changes. Stress and fear affect the decreased release of gonadotropin releasing hormone (GnRH) which in turn influences the secretion of estrogen and progesterone. Maladaptive stress activates the anterior pituitary axis (HPA), which causes the hypothalamus to secrete corticotropin-releasing hormone (CRH). CRH has a negative effect on the secretion of GnRH, leading to delayed sexual maturation.[17] In our study, we found no significant relationship between psychosocial dysfunction and menarche age (P=0.28).

A limitation of our study was that the history of audio-visual media exposure was only obtained from questionnaires. As such, the answers may have been subject to recall bias. Also, the nutritional status assessment was done by anthropometric examination; subjects were not asked directly about their daily diet. In addition, socio-economic status was assessed according to parental income divided into low and high socio-economic status, but more

than 90% of subjects were in the high category. Furthermore, psychosocial dysfunction was assessed by the PSC 35, no specific psychological examination was performed. Nevertheless, the results of our study reasonably describe some factors that could affect age at menarche in the Medan population, in particular. We also found an earlier mean age at menarche than in past decades.

Conflict of Interest

None declared.

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Original Article

Mantoux tests of children in household contact with adult acid fast bacilli-positive or -negative pulmonary tuberculosis

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Abstract

Background Tuberculosis (TB) is the leading cause of mortality and morbidity in developing countries. In children, the major source of TB transmission is adults with pulmonary TB who have acid fast bacilli (AFB)-positive sputum. However, tuberculosis infection can also occur in children in household contact with adults who have AFB-negative pulmonary TB.

Objective To compare Mantoux test results and induration diameters in children with adult pulmonary TB household contact who were either positive or negative for AFB, and to assess for possible associations between Mantoux test results with age, family income, and house ventilation in both groups.

Methods A cross-sectional study was conducted from January to March 2014. Mantoux test was performed in children aged 3 months to 15 years who had household contact with either AFB-positive or -negative adult pulmonary TB patients.

Results A total of 106 children were enrolled in the study. All subjects had household contact with adult pulmonary TB patients who were either AFB-positive (54 children) or AFB-negative (52 children). Mean Mantoux test induration diameters were significantly different between groups (10.9 (SD 6.55) mm *vs.* 6.2 (SD 5.91) mm, respectively; P=0.001). In addition, there was significantly higher risk of positive Mantoux test in children in contact with adult AFB-positive TB patients than in the AFB-negative group (OR 5.66; 95%CI 2.36 to 13.59; P=0.0001). However, there were no significant differences in positive Mantoux test results in each of the AFB-positive and -negative groups, with regards to age, family income, or house ventilation.

Conclusion Mean Mantoux test induration diameter in children who had household contact with AFB-positive adults is significantly larger than that of the AFB-negative group. Positive Mantoux test results in children are associated with AFB-positive adult TB in the household. There is no association between positive Mantoux test results and age, family income, or house ventilation in both groups. **[Paediatr Indones. 2017;57:310-5 ; doi: http://dx.doi.org/10.14238/pi57.6.2017.310-5].**

Keywords: *tuberculosis; children; acid fast bacilli; household contact*

Tuberculosis (TB) is a disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) and is one of the leading causes of mortality and morbidity in developing countries.[1] Indonesia ranks fourth worldwide after India, China, and South Africa, as the country with the highest TB burden.[2] In 2011, TB prevalence in Indonesia was 281 per 100,000 people and TB incidence reached 187 per 100,000 people. The mortality rate was 27 per 100,000 people.[2,3] Tuberculosis in Indonesian children was 9% of total TB cases.[3]

This study was presented at the *Kongres Nasional Ilmu Kesehatan Anak XVI/ KONIKA XVI* (The 16th National Congress of Child Health), Palembang, August 25–28, 2014.

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Several factors facilitate TB infection spread to children such as contact with adult TB patients, living in endemic areas, poverty, and poor sanitation.[4] In terms of prevalence, more children suffer from TB infection and TB disease after contact with adult patients than the general population. The risk for infection in children increases when they have household contact with adult pulmonary TB patients with sputum positive for acid fast bacillus (AFB).[5,6] Infection could also occur with AFB-negative adult pulmonary TB household contacts.[7] Tuberculin test is used to detect *M. tuberculosis* infection.[4] Children in contact with adults who had positive TB smears and active TB infection had larger Mantoux test induration diameters.[8]

The purpose of this study was to compare Mantoux test results and induration diameters in children with AFB-positive or negative adult TB patient household contact. We also assessed for possible associations between Mantoux test results with age, family income, and house ventilation, in both the AFB-positive and -negative groups.

Methods

This cross-sectional study was conducted in private practices of pulmonary physicians and the Institute for Lung Health Society (*Balai Kesehatan Paru Masyarakat, BKPM*) Medan, North Sumatra from January to March 2014. The subjects were children aged 3 months to 15 years who had household contact with adult pulmonary TB patients. Children with an immunocompromized state (undergoing treatment with long-term corticosteroid therapy, cytotoxic drugs, or other immunosuppressive drugs), malnutrition, measles, mumps, severe tuberculosis, abdominal typhoid, or malignant disease were excluded. Children who underwent Mantoux test within the 2 weeks prior or had polio or measles immunization in the 6 weeks prior were also excluded. This study was approved by the Research Ethics Committee of the University of Sumatera Utara Medical School, Medan. Subjects' parents provided written informed consent.

Adult pulmonary TB patients were identified by their medical records. Pediatric subjects were divided into two groups based on contact history, either AFB-positive or negative adult TB patients. Subjects' characteristics and information were obtained from questionnaires that were filled out by parents. Mantoux test was performed on all subjects, with 0.1 mL of 2TU PPD RT-23 intradermally on the volar surface of the left forearm. The induration was measured after 48 to 72 hours. Mantoux test was considered to be positive if the induration diameter was ≥ 10 mm.

Household contact was defined as a child living in the same home with an adult pulmonary TB patient for at least 3 months. Adults were diagnosed with pulmonary TB based on suggestive symptoms and signs, confirmed by either presence of TB bacilli on Ziehl-Neelsen staining of sputum (referred to as AFB-positive) or diagnostic chest radiography in the absence of TB bacilli in sputum (AFB-negative). Family income was assessed by monthly parental income, compared to the or minimum wage (*Upah Minimum Kota, UMK*) of Medan municipality, North Sumatera, and classified as higher or lower than the UKM. House ventilation was assessed by measuring the house surface area and ventilation surface area. House ventilation was considered to be good if the ventilation area size was more than 10% of the house surface area, and not good for < 10%.

The collected data were processed, analyzed, and presented using *SPSS 16 version* software. Chi-square test was used to assess for an association between a history of TB contact and Mantoux test results. Independent T-test was used to analyze for differences in the induration diameter size of the Mantoux test. Significance was set at $P<0.05$ with 95%CI.

Results

A total of 106 children were admitted to the study, of whom 54 children had contact with AFB-positive adults and 52 children had contact with AFB-negative adults with pulmonary TB. A total of 67 adults with TB had household contact with children, 33 AFB-positive and 34 AFB-negative. Characteristics of pediatric subjects in both groups are shown in **Table 1**. The mean age, sex, weight, and height of between the AFB-positive and -negative groups were slightly different. The majority of parents had graduated from senior high school and were self-employed. Family income less than UMK for subjects in the

AFB-positive and -negative groups were 64.8% and 53.8%, respectively. In the AFB-positive group, the majority of subjects' household TB contact was fathers. However, in the AFB-negative group, the same percentage of children had fathers and mothers as the contact source (42.3% for each). No significant difference was found in both groups.

Mantoux test induration diameters ranged from 0 to 25 mm in the AFB-positive group and 0 to 27 mm in the AFB-negative group. **Table 2** shows that the mean induration diameter of the Mantoux test of the AFB-positive group was significantly greater than that of the AFB-negative group (P=0.001).

Positive Mantoux test results were found in 31 (57.5%) children with AFB-positive household contact. However, in the AFB-negative group, 10 (19.2%) children had positive and 42 (80.8%) children had positive Mantoux test results. Chi-square test revealed significant differences in Mantoux test results in both groups (P=0.0001) (**Table 3**).

Table 4 shows the relationships between the Mantoux test results in the AFB-positive and -negative groups with age, family income, and house ventilation, none of which had significant associations.

Table 1. Characteristics of subjects

Characteristics	AFB-positive (n=54)	AFB-negative (n=52)
Age, n (%)		
≤5 years	10 (18.5)	15 (28.9)
>5 years	44 (81.5)	37 (71.1)
Sex, n (%)		
Male	23 (42.6)	28 (53.8)
Female	31 (57.4)	24 (46.2)
Mean weight (SD), kg	27.5 (11.93)	24.5 (12.21)
Mean height (SD), cm	127.5 (24.43)	119.6 (24.16)
Nutritional status, n (%)		
Wasted	19 (35.2)	16 (30.8)
Normoweight	33 (61.1)	34 (65.4)
Overweight	2 (3.7)	2 (3.8)
Paternal education, n (%)		
Elementary school	4 (7.5)	1 (2.0)
Junior high school	6 (11.3)	9 (18.4)
Senior high school	31 (58.5)	28 (57.1)
University	12 (22.7)	11 (22.4)
Maternal education, n (%)		
Elementary school	2 (3.7)	4 (7.7)
Junior high school	13 (24.1)	10 (19.2)
Senior high school	36 (66.7)	30 (57.7)
University	3 (5.5)	8 (15.4)
Family income, n (%)		
< UMK	35 (64.8)	28 (53.8)
≥ UMK	19 (35.2)	24 (46.2)
House ventilation, n (%)		
Not good	27 (50.0)	22 (42,3)
Good	27 (50.0)	30 (57.7)
Contact source, n (%)		
Father	26 (48.1)	22 (42.3)
Mother	18 (33.3)	22 (42.3)
Sibling	3 (5.6)	2 (3.8)
Grandfather/grandmother	5 (9.3)	6 (11.5)
Uncle/aunt	2 (3.7)	0

Table 2. Mantoux test induration diameters

	Positive, n(%)	Negative, n(%)	Mean diameter (SD)	Mean difference (SD)	95% CI	P value
AFB-positive (n=54)	31 (57.4)	23 (42.6)	10.9 (6.55)	4.84	2.45 to 7.24	0.001
AFB-negative (n=52)	10 (19.2)	42 (80.8)	6.2 (5.91)			

Table 3. Mantoux test results

Contact	Mantoux test		OR	95%CI	P value
	Positive, n(%)	Negative, n(%)			
AFB-positive	31 (57.4)	23 (42.6)	5.66	2.36 to 13.59	0.0001
AFB-negative	10 (19.2)	42 (80.8)			

Table 4. Mantoux test results in children by AFB group and age, family income, and house ventilation

Variables	AFB-positive				AFB-negative			
	Mantoux tes		OR 95%CI	P value	Mantoux test		OR 95%CI	P value
	Positive (n=31)	Negative (n=23)			Positive (n=10)	Negative (n=42)		
Age, n (%)								
≤5 years	4(12.9)	6(26.1)	0.42	0.297	2(20.0)	13(31.0)	0.56	0.704
>5 years	27(87.1)	17(73.9)	(0.10 to 1.71)		8(80.0)	29(69.0)	(0.10 to 2.99)	
Family income, n(%)								
<UMK	22(71.0)	13(56.5)	1.88	0.272	7(70.0)	21(50.0)	2.83	0.291
≥ UMK	9(29.0)	10(43.5)	(0.61 to 5.83)		3(30.0)	21(50.0)	(0.64 to 12.44)	
House ventilation, n(%)								
Not good	15(48.4)	12(52.2)	0.86	0.783	5(50.0)	17(40.5)	1.47	0.725
Good	16(51.6)	11(47.8)	(0.29 to 2.53)		5(50.0)	25(59.5)	(0.37 to 5.87)	

Discussion

We found that the mean induration diameter of the Mantoux test in children in household contact with adult AFB-positive TB patients was significantly higher than that of the AFB-negative group, similar to a Spanish study.[8] Household contact with adult TB patients is a risk factor for TB infection in children. A study in Laos reported that the risk of TB infection in children with TB contact is greater for children in contact with smear-positive TB patients.[9] Also, a Turkish study showed that the risk of TB infection in children was higher for those in contact with adults who were smear-positive and had cavities in the lungs on chest x-ray.[10] Furthermore, we found significantly more positive Mantoux test results in the AFB-positive group than in the AFB-negative group, with an odds ratio of 5.6. This result was greater than that of an Indian study, which reported an odds ratio of 3.2.[5] A Manado study also showed that household contact with AFB-positive adult TB was a risk factor for TB infection in children.[11]

Parents, both fathers and mothers, were the majority contact source of TB in our study. A Brazilian study showed fathers to be the main contact,[12] while a Pakistani study showed mothers to be the main contact, because they spent more time with the children than other family members.[13] Having contact with more than one TB patient in a house also increased the risk of TB illness.[12] In our study we found only one child with more than one adult TB household contact. The prevalence of TB infection in children having household contact with AFB-negative adult pulmonary TB in this study was 19.2%. This prevalence was higher than in West Java, with a 10% prevalence for TB infection and 16% for TB disease.[7] A study in Nigeria showed positive tuberculin tests in 49% and 16% of children with AFB-positive and -negative adult TB household contacts, respectively,[6] while a study from India reported 46.4% and 21.3%, respectively.[5]

The incidence of TB infection increases in accordance with age. A systematic review showed that TB disease was more commonly found in children less than 5-year-old, but TB infection was more commonly found in older children.[14] We noted that positive Mantoux test results were more common in children older than 5 years, regardless of the AFB status of their household contact.

Density and poor ventilation increase the probability of TB infection. People living in crowded areas or with poor ventilation have higher risk of TB exposure. The risk of a positive tuberculin test in subjects with adult TB household contact increased as house density increased. A Bangkok study showed that children living in dense environments had five times higher risk of being infected with TB than children living in less dense environments. The residential area also contributes to the spread of TB due to environmental conditions, poor ventilation, poor hygiene, and crowding.[15] Unfortunately, we did not assess house density. We only assessed house ventilation, which was not associated with Mantoux test results.

Tuberculosis has been associated with poverty, as reflected by low family income. Poor socio-

economic condition may increase infection through poor nutrition and high population density. Studies in Great Britain, the Philippines, and Cambodia showed that the incidence of TB was closely related to poverty.[16-18] However, we found no such relationship between low income and TB infection. This difference may be due to poverty assessment methods. We used the minimum regional wage (UMK) in Medan, where the study was conducted, as a cut-off point for household income. Similarly, a study in West Java also found no relationship between family income and positive tuberculin test in children with adult pulmonary TB household contact.[19]

Some limitations were noted in the study, such as not performing TB scoring and defining infection based on Mantoux test reaction, regardless of presence/absence of symptoms. In addition, history of BCG immunization weren't assessed. Further study is required to determine TB disease in children and assess the risk factors for TB infection in children.

In conclusion, mean induration diameter of Mantoux test in children with AFB-positive adult TB patient household contact is significantly larger than in children in the AFB-negative group. In addition, a greater number of positive Mantoux test results are associated with AFB-positive household contact. There are no associations between positive Mantoux test results and age, family income, or house ventilation in either group.

Conflict of Interest

None declared.

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Original Article

Maternal attitude and child interest in various play activities before and after mother-child play sessions

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Abstract

Background Play stimulates children's growth and development. When mothers and their children play, a positive attitude from the mother and adequate interest from the child is required. Little is known about the play activities that effectively stimulate such positive maternal attitude and child interest.

Objective To assess for associations between various play activities with maternal attitude and child interest before and after mother-child play sessions.

Methods Pre-post intervention questionnaires were distributed to mothers before and after playing with their children. Children were aged 1-5 years, from two play sites (in Surabaya and Makassar), and included using purposive sampling. Eight types of toys/play activities were provided. The allocated time for answering the 17-question survey was 15 minutes. Average scores before and after the mother-child play sessions were analyzed using paired T-test.

Results We collected 264 valid questionnaires, 235 in Surabaya and 29 in Makassar. Improvement of maternal attitude after the mother-child play session was found in 132 mothers [mean diff. 0.07 (SD 0.42); 95%CI -0.117 to -0.015; P=0.011]. Play activities with significant improvements in maternal attitude were jigsaw puzzle [mean diff. 0.09 (SD 0.66); 95% CI 0.007 to 0.167;P= 0.033), Lego blocks [mean diff.-0.10 (SD 0.69); 95%CI -0.186 to -0.018; P=0.017], mini-gardening [mean diff. -0.15 (SD 0.75); 95%CI -0.238 to -0.057; P=0.002], sandbox [mean diff.-0.24 (SD 0.83); 95%CI -0.339 to -0.138; P < 0.001], fishing [mean diff. -0.17(SD 0.68); 95%CI -0.253 to -0.088; P < 0.001], and animal figurines [mean diff. -0.21(SD 0.75); 95%CI -0.3 to -0.117;P <0.001]. Improvement of child interest was found in 161 children [mean diff. 0.20 (SD 0.52); 95%CI -0.264 to -0.116; P<0.001]. Play activities with significant improvements in child interest were jigsaw puzzle, Lego blocks, origami, mini-gardening, fishing, and animal figurines.

Conclusion Some mother-child play activities, but not all, significantly improve both maternal attitude and child interest toward play. **[Paediatr Indones. 2017;57:316-22 ; doi: http://dx.doi.org/10.14238/pi57.6.2017.316-22]**.

Keywords: *attitude; children; interest; mother; playinghousehold contact*

Children's growth and development are determined by the quality of the nutrition, stimulation, and family affection that they receive. Regular play stimulates the development of many important functions, such as visual and auditory function, cognitive and verbal skills, gross and fine motor skills, as well as problem-solving capability. Stimulation through regular play also develops children's personal interests, knowledge, cognition, creativity, and independence.[1,2,3] Children benefit from repetious play as they have the opportunity to visualize, and

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consolidate methods and information.[3] Beneficial play requires family support, especially from mothers, who have an important role in motivating children to play. Maternal attitude towards mother-child play is related to maternal knowledge level and the children's response to a particular play activity.[4,5]

Playing with children is essential for both mother and child. Mothers provide children with necessary support and rewards. Children feel secure and comfortable because of maternal protection and guidance. This experience develops a child's confidence, courage, and creativity to utilize their playground.[6] Benefits of play are also found in mothers because they recognize their children's interest and capabilities, resulting in stronger emotional bonds. In turn, mothers can better select games or toys suitable for their children's interest and talents. This bonding improves the mother's ability to play with her child.[5,6,7] Other factors associated with maternal attitude, besides knowledge, include level of education, information resources, number of children, and socio-economic status.[8,9] Mothers who work as the sole breadwinner of the family may not give adequate affection to maximally support their children's growth and development.[9] A large number of children in the family also affects the distribution of affection and the play time for each child. In contrast, mothers with only one child probably have less experience in parenting.[10]

This study was done with the aim of assessing for associations between types of play activities and maternal attitude as well as child interest, through questionnaires filled by mothers before and after play sessions.

Methods

This pre-and post-interventional study was conducted from August to October 2016 at two, purpose-built, play sites in Surabaya and Makassar. Subjects were recruited with purposive sampling. The inclusion criteria were mothers with children aged 1 to 5 years, both mothers and children were engaged in at least 6 out of 8 play activities provided at the play site, and were willing to fill the questionnaires. All participants were provided with written informed consent. Only mothers with normal pregnancies and birth histories were included. Exclusion criteria were mothers who didn't fully complete the questionnaires or those whose children could not complete the play activity as expected.

Demographic characteristics were classified into several categories. Participants' monthly incomes were classified into 3 categories: <Rp 2,000,000; the national minimum wage of Rp 2,000,000 to Rp 5,000,000, and > Rp 5,000,000.11 Their educational backgrounds were classified as low for junior high school graduates or below, middle for senior high school graduates, or high for post-high school/ university educated and above. Other classifications were number of children in the family, employment status, and sole nurturer of children.

Indonesian language questionnaires used in this study were designed by our study team and validated at the Kiara Clinic, Department of Child Health, University of Indonesia Medical School/Cipto Mangunkusumo General Hospital, a teaching hospital. Questionnaires included demographic characteristics and questions regarding maternal attitude and child interest towards various types of play activities. The maternal attitude towards playing activities questions were scaled from 0 to 3, with 0 for "not essential," 1 for "less essential," 2 for "essential," and 3 for "very essential." For questions about child interest, 0 referred to "not interested," 1 for "less interested," 2 for "interested," and 3 for "very interested." The allocated time for filling the questionnaire was 15 minutes for 17 questions.

The play activities assessed in this study were (1) coloring, (2) jigsaw puzzle, (3) Lego blocks, (4) origami, (5) mini-gardening, (6) sandbox, (7) fishing, and (8) 3D animal figurines. The time allocation for each activity was 10-15 minutes. Questionnaires were collected and evaluated to obtain the mean scores of maternal attitude and child interest before and after the play session. For mean scores of each play activity, child interest score of 2.26 to 3 was considered good, 1.51-2.25 fair, 0.76-1.5 poor, and under 0.75 no interest. For mean scores of each play activity, maternal attitude score of 2.26 to 3 was considered to be good, 1.51-2.25 fair, 0.76-1.5 poor, and under 0.75 not favorable . Mean scores were then analyzed for significant changes by paired T-test using *SPSS version 22.0 for Windows*.

This study was approved by the Health Research Ethics Committee, University of Indonesia Medical School/Cipto Mangunkusumo General Hospital, Jakarta.

Results

Four hundred fifty questionnaires were distributed, 350 in Surabaya and 100 in Makassar. Of these, 367 mother-child pairs were recruited (310 in Surabaya and 57 in Makassar). However, only 264 (71.9%) mothers completed the questionnaires as required, 235 from Surabaya and 29 from Makassar.

Table 1. Demographic characteristics of subjects

Characteristics	Criteria	Value
Maternal age, years	Range Mean (SD)	20-54 31 (4.59)
Educational background, n(%)	Low Middle High	14 (5.3) 128 (48.5) 122 (46.2)
Employment, n(%)	Stay-at-home mom Working mom	150 (56.8) 114 (43.2)
Family monthly income, n(%)	< Rp 2,000,000 Rp 2,000,000 - Rp 5,000,000 > Rp 5,000,000	59 (22.3) 165 (62.5) 40 (15.2)
Nurturing child by herself, n(%)	Yes No	199 (75.4) 65 (24.6)
Number of children, n(%)	1 2 3 or more	169 (64) 73 (27.7) 22 (8.3)

The mean maternal age was 31 (SD 4.59) years, ranging from 20 to 54 years. Almost half of the subjects had high educational background (122; 46.2%), while 128 subjects (48.5%) had middle, and 14 subjects (5.3%) had low educational background. The majority (56.8%) were stay-at-home mothers. Most subjects had a household income above the national minimum wage (77%) and most nurtured their children by themselves (75.4%). In addition, most mothers had only 1 child (64%) (**Table 1**).

In both Surabaya and Makassar, television is the predominant source of information, with 43% and 58% respectively, and an overall 44.7% of subjects. In contrast with subjects from Surabaya, subjects from Makassar barely relied on information from doctors/midwives/nurses. Information sources were understood by most mothers (85.2%), with only 37 mothers (14.8%) admitting to not understanding their sources of information in the questionnaire (**Table 2**).

Child interest in the play activities was scored in pre- and post-intervention questionnaires completed by mothers according to their observations before and after the play sessions. As shown in **Table 3**, according to maternal observations before the play sessions, children had only a 'fair' level of interest towards provided activities. The overall mean general score for children interest before playing session in the provided activities was 1.96 (SD 0.53). After playing session the mean general score was increased to 2.16 (SD 0.58), however this score was still considered 'fair'.

Table 2. Distribution of information sources on the importance of playing with children

Sources of information	Site			Understanding	
	Surabaya (n=235)	Makassar (n=29)	Total (N=264)	Understood (n=225)	Not understood (n=34)
Television, n(%)	101 (43.0)	17 (58.6)	118(44.7)	103 (45.8)	15 (44.1)
Magazine/newspaper, n (%)	5 (2.1)	2 (6.9)	7 (2.7)	7 (3.1)	0
Shopkeepers, n (%)	7 (3)	1 (3.4)	8 (3)	5 (2.2)	3 (8.8)
Mobile phone/social media, n (%)	64 (27.2)	3 (10.3)	67 (25.4)	57 (25.3)	10 (29.4)
Lecture/seminar, n (%)	10 (4.3)	2 (6.9)	12 (4.5)	10 (4.4)	2 (5.9)
Doctors, n (%)	7 (3.0)	0	7 (2.7)	5 (2.2)	2 (5.9)
Nurses/midwives, n (%)	5 (2.1)	0	5 (1.9)	5 (2.2)	0
Friends, n (%)	25 (10.6)	2 (6.9)	27 (10.2)	25 (11.1)	2 (5.9)
Others, n (%)	8 (3.4)	0	8 (3)	8 (3.6)	0
No source, n (%)	3 (1.3)	2 (6.9)	5 (1.9)	N/A	N/A

Six play activities had significantly improved mean child interest scores after the play sessions with such increase: (1) jigsaw puzzle [0.16 (SD 0.95)], (2) Lego blocks [0.21 (SD 1.03)], (3) origami [0.25 (SD 0.99)], (4) mini-gardening [0.37 (SD 1.13)], (5) fishing [0.29 (SD 0.96)], and (6) animal figurines [0.16 (SD 0.92)]. Overall, we found improved interest in 161 children (61%) with mean difference of 0.20 (SD 0.52); 95%CI -0.264 to -0.116; P<0.001]. This finding demonstrated that playing with children was beneficial in enhancing children to play, introducing new points during the games, and expanding children's interest toward various playing activities (**Table 3**).

In the questionnaire, maternal attitude questions were increased concomitantly with the child interest questions. The mean overall maternal attitude on the importance of play increased with mean difference of 0.07(SD 0.42); (95%CI -0.117 to -0.015; P<0.05). An improvement in maternal attitude was found in 132 (50%) mothers after the play sessions. This finding is shown in **Table 4**.

Significant improvements of mean maternal attitude scores were found in the following play activities with such increase: (1) jigsaw puzzles [0.09 (SD 0.66)], (2) Lego blocks [0.10 (SD 0.69)]; (3) mini-gardening [0.15 (SD 0.75)]; (4) sandbox [0.24

Table 3. Mean child interest scores for each activity before and after the mother-child play sessions, based on maternal observations

No	Rank the child's interest in	Mean score (SD)		Mean difference (SD)	95% CI	P value
		Before	After			
1	Coloring pictures	2.14 (0.89)	2.23 (0.83)	-0.09 (0.92)	-0.202 to 0.02	0.109
2	Jigsaw puzzles	2.02 (0.92)	2.18 (0.84)	-0.16 (0.95)	-0.274 to -0.44	0.007
3	Lego block	1.84 (1.03)	2.06 (0.91)	-0.21 (1.03)	-0.337 to -0.087	0.001
4	Origami	1.66 (0.94)	1.9 (0.96)	-0.25 (0.99)	-0.366 to -0.127	< 0.001
5	Mini-gardening	1.54 (1.02)	1.91 (0.98)	-0.37 (1.13)	-0.509 to -0.234	< 0.001
6	Sandbox	2.18 (0.95)	2.25 (0.95)	-0.07 (1.00)	-0.194 to 0.05	0.247
7	Fishing	2.24 (0.94)	2.53 (0.74)	-0.29 (0.96)	-0.408 to -0.176	< 0.001
8	Animal figurines	2.03 (1.00)	2.2 (0.94)	-0.16 (0.92)	-0.274 to -0.052	0.004
	General score for child interest	1.96 (0.53)	2.16 (0.58)	-0.20 (0.52)	-0.264 to -0.116	< 0.001

Table 4. Mean maternal attitude score for each play activity before and after the mother-child play sessions

No	Rank the child's interest in	Mean score (SD)		Mean difference (SD)	95% CI	P value
		Before	After			
1	Coloring pictures	2.48 (0.53)	2.44 (0.55)	0.04 (0.61)	-0.032 to 0.115	0.265
2	Jigsaw puzzles	2.33 (0.54)	2.43 (0.58)	0.09 (0.66)	0.007 to 0.167	0.033
3	Lego block	2.11 (0.66)	2.21 (0.68)	-0.10 (0.69)	-0.186 to -0.018	0.017
4	Origami	2.18 (0.61)	2.17 (0.67)	0.00 (0.73)	-0.084 to 0.092	0.933
5	Mini-gardening	2.05 (0.70)	2.19 (0.68)	-0.15 (0.75)	-0.238 to -0.057	0.002
6	Sandbox	1.81 (0.84)	2.05 (0.81)	-0.24 (0.83)	-0.339 to -0.138	< 0.001
7	Fishing	2.02 (0.73)	2.19 (0.67)	-0.17 (0.68)	-0.253 to -0.088	< 0.001
8	Animal figurines	1.87 (0.85)	2.08 (0.79)	-0.21 (0.75)	-0.300 to -0.117	< 0.001
9	Playing with your child	2.63 (0.47)	2.77 (0.58)	0.14 (0.68)	0.058 to 0.223	0.001
	General score for child interest	2.19 (0.41)	2.26 (0.47)	-0.07 (0.42)	-0.117 to -0.015	0.011

P value <0.05 indicates statistical significance

(SD 0.83)] (5) fishing [0.17 (SD 0.68)], and (6) animal figurines [0.21 (SD 0.75)]. Before mothers engaged in play sessions with their children, their attitude was low for those 6 play activities, but increased significantly after play, despite the score was still fair. This increase was also happened in their attitude on playing together with child (**Table 4**).

The overall score for maternal attitude towards the importance of play was good before the play session and increased after the play session [before: 2.19 (SD 0.41); after 2.26 (SD 0.47); 95%CI of mean difference -0.117 to -0.015; P 0.011]. This result was then analyzed using Spearman's analysis to see its correlation with prior knowledge and understanding level of the mothers with P value 0.021 and Spearman's correlation score 0.142 .

Discussion

The parent-child bond can be strengthened by play. Play activities, mainly engaged in by mothers with their children, have a positive impact on optimal child growth and development.[5-7,10] Numerous types of play activities are benficial. Mothers' observing their children playing can have a positive effect on their own attitude towards play, depending on the type of activity. Their experience of engaging in an activity changes their perception and attitude towards that activity. A prior study on breastfeeding mothers showed that maternal attitude towards breastfeeding changed positively after they breastfed their own children.[12] From our questionnaire-based study, we found that maternal attitude towards play changed positively after mothers played with their child by themselves ($P<0.05$). Maternal attitude was also affected by their prior understanding about the benefit of play, which shown in this study (**Table 2**). However, the correlation was very weak (Spearman's correlation 0.14). In contrast, a study also done in Indonesia about maternal attitude on breastmilk and complementary food for babies showed that prior maternal knowledge highly affected their attitude.[13] The influence of prior knowledge was also found in other studies about maternal attitude towards vaccines and vaccination.[14] Although, in general, maternal attitude scores improved, there were not significant changes for picture coloring and origami folding. Origami has been shown to have positive behavioral and cognitive impacts on children, as they have a calmer and friendlier attitude after making origami, as well as better perception of mathematics.[15,16]

Despite the finding in maternal attitude towards origami, child interest in origami significantly increased from before to after the play session, based on maternal perceptions. Significant increases in child interest were also found in Lego blocks, jigsaw puzzles, mini-gardening, fishing, and animal figurines provided at the play site ($P<0.05$). Lego blocks, jigsaw puzzles, and origami are easy-to-implement, home-based activities that can be done by parents to introduce mathematical perception to their children in a fun, engaging manner. Doing these activities with their mothers, children may develop an early interest of mathematics, as well as other skills and knowledge.[17] A prior study in preschoolers, for instance, showed that puzzles improve spatial skills. Children who assembled puzzles with their parents had better spatial skills compared to those who did not. They also received more language input from their parents.[18]

Other activity that is beneficial for developing children's abilities is sandbox playing. Sandbox playing, mostly found in public playground, is an ideal place to develop imaginative skills, social and community skills, as well as spatial and cognitive skills. Children who met each other in the playground will interact and develop relationship between them. Mother would be helpful to assist children playing in the sandbox.[19] In this study, however, playing with children in the sandbox didn't show a significant change on children's interest. There was an increase of average score about 0.07 (SD 1) point, but this finding was not statistically significant ($P>0.05$, 95%CI of mean difference -0.194 to 0.05). Parent's involvement might be affecting, knowing that parents are the chief facilitator of play in their children.[20] Other factors that also affecting is the site where the study was held. Makassar and Surabaya are port cities which makes children might be familiar to playing sand on the beach. Different playing environment have an impact on children's play activities, therefore may be it is the reason the increase of average score in playing sandbox not so significant.[21]

Mother-child play activities had significant associations ($P<0.05$) with increased child interest, with the exception of picture coloring and playing in

the sandbox. Children's play activities also increased maternal attitude in a positive manner on the importance of playing with their children, especially engaging in activities such as Lego blocks, jigsaw puzzles, mini-gardening, sandbox, fishing, and animal figurines. Therefore, mothers should be encouraged to play more with their children, not limited to the play activities in this study, to gain benefits for both mothers and children.

A limitation of this study was the lack of an exact time limit for each play activity, such that the amount of time spent by each child may have differed. Further study needs to be done to assess what would constitute an adequate time for this observation. Other types of play activities may also be done in future studies to test and compare their benefits.

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Conflict of Interest

None declared.

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Original Article

Risk factors of soil-transmitted helminth infection among elementary school students

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Abstract

Background Helminth infection remains a health problem, especially in school-aged children. Mass eradication programs with a single dose of anti-helminthic drugs were employed by the local government in some endemic areas in Bali. However, the effectiveness of the programs has not been well evaluated.

Objective To investigate prevalence and possible risk factors of helminth infection, including nutritional status, in elementary school students from endemic areas who participated in mass eradication programs.

Methods This cross-sectional study involved 126 students from Elementary School No. 3 Gegelang, Karangasem, Bali, a location that had recently undergone a mass eradication program. Diagnoses were based on direct smear examination of fecal specimens. Information on suspected risk factors and nutritional status were collected by questionnaire and anthropometric measurement, respectively. Statistical analyses included Chi-square and odds ratio, using *SPSS v21 software*.

Results The prevalence of helminth infection was 31.7% with etiologies of *Trichuris trichuria* (75%), *Ascaris lumbricoides* (17.5%), or both infections (7.5%). Habits of not using footwear [OR=4.88; 95%CI 1.15 to 20.65], not keeping nails trimmed [OR=3.33; 95%CI 1.07 to 10.37], and absence of a proper toilet [OR=4.31; 95%CI 1.93 to 9.64] were found to be significant risk factors for helminth infection. However, we found no significant association between helminth infection and nutritional status, although a considerable number of students had less than normal reference values, in terms of weight, height, and BMI for age.

Conclusion The prevalence of helminth infection continues to be high, with personal hygiene and sanitation as significant risk factors. History of mass eradication programs did not confer an effective protection against helminth infection. **[Paediatr Indones. 2017;57:295-302 ; doi: http://dx.doi.org/10.14238/pi57.6.2017.295-302]**.

Keywords: *helminth infection; soil-transmitted helminth; Trichuris trichuria; Ascaris lumbricoides; school-aged children*

Soil-transmitted helminth (STH) infection is a neglected tropical disease which causes a significant public health burden in developing countries.[1] Among the most common infections, STH affects the poorest and most deprived communities worldwide.[2] The STH is associated with low income, poor personal hygiene and environmental sanitation, limited access to clean water, tropical climate, and low altitude.[3] A 2010 estimate suggested that 438.9 million people were infected with hookworm, 819 million with *Ascaris lumbricoides*, and 464.6 million with *Trichuris trichuria*, with 70% of the cases occurring in Asia.[4] The STH infection, either by single or mixed agents, rarely causes death. However,

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the infection affects nutritional intake, digestion, absorption, and metabolism of the host.[2,5]

The STH predominantly affects school-aged children. Moreover, this population is the most prone to detrimental effects of parasites.[5,6] These effects include nutritional deficiency, anemia, as well as impaired physical growth and mental development.[5] All of these detriments lead to school absenteeism and poor school performance.[1,5] The *World Health Organization* (WHO) advocates anti-helminthic preventive therapy as the main strategy to control such morbidity in countries with high STH endemicity.[7] However, reinfection after successful treatment, especially in the absence of targeted hygiene education and measures to improve sanitation and water supplies, is the major obstacle to mass drug administration programs.[1] Indeed, studies in China have shown that reinfection rates after treatment with albendazole were around 75% and 83%, in 4 and 6 months, respectively.[8,9] In Indonesia, mass drug administration by the local government is a common practice in endemic areas. The program consists of giving a single dose of anti-helminthic to school-aged children every 6 or 12 months. However, effectiveness and reinfection rates of children who received treatment has not been well evaluated.

Nutritional status has a close relationship with infectious disease. Poor nutritional status increases general susceptibility to infection, while infections negatively impact nutritional status.[5,8] This interaction results in a vicious cycle of under-nutrition and infection.[8] Although evidence of increased susceptibility during under-nutrition to STH infection has been inconclusive, it is worth considering nutritional intervention to complement mass drug administration in the future.[8]

Prevalence, risk factors, and nutritional impact of STH infection could vary with localities, therefore, such information is vital to guide policy makers in designing a more focused, preventive approach to control the disease. However, local data regarding this parameter is still lacking, despite the practice of mass drug administration for school-aged children in endemic areas. Therefore, we aimed to estimate prevalence of STH infection, associated risk factors, and nutritional status of students in an area with routine mass drug administration.

Methods

A cross-sectional study was conducted on September 2016 in Elementary School (SD) No. 3 Gegelang, Karangasem Regency, Bali Province. Subjects were collected by total sampling. The 126 participants and their parents provided informed consent. Subjects submitted fecal specimens for examination. This study was approved by the Ethics Committee of Udayana University Medical School, Denpasar, Bali.

Subjects' baseline data were obtained from interviews, questionnaires, and anthropometric measurements. A clean, dry, and leak-proof 60-mL urine pot, pre-labelled with the subject's name and an identification number, was issued to each recruited student. Fecal examination was done by trained analysts using a direct smear (direct wet mount) method, on site, to diagnose STH infection. Body weight (BW) was measured to a 0.1 kg precision, with the child wearing minimal clothing and no shoes. Body height was measured to a 0.1 cm precision, with the child standing up straight.

Nutritional status classification was based on Z-score of body-weight-for-age, height- for-age and BMI-for-age. Height-for-age Z-score was used as an indicator of chronic malnutrition (stunting). Weight-for-age Z-score was used as the general indicator of child general nutritional status (underweight). BMI-for-age Z-score was used as an indicator of the child being too thin for his/her height. Nutritional status was analyzed based on the *WHO 2007* reference chart.[10] Students with Z-scores below −2 standard deviations (SD) of the WHO reference population median values for weight-, height-, and BMI-for-age were considered to be underweight, stunted, or thin, respectively. Z-score cut-off of < −3 SD was considered to be a more severe condition for respective categories.

Finally, demographic information, completed questionnaires, anthropometric data, and test results were analyzed using *SPSS software version 21* (SPSS Inc., Chicago, IL). Chi-square and odds ratio were used to analyze for relationships between STH infection status and suspected risk factors, as well as STH infection status and nutritional status. Logistic regression was used to calculate adjusted odds ratios and corresponding 95% confidence intervals for those variables revealed to be significantly associated by

Chi-square analysis. Results were considered to be statistically significant for P values <0.05.

Results

Subjects were recruited from Elementary School No. 3 Gegelang, Karangasem, Bali. The school location is about 42 kilometers northeast of Denpasar. A total of 126 students completed interviews, weight and height measurements, and provided fecal specimens. There were 67 (53.2%) males and 59 (46.8%) females. Students ranged in age from 6 to 16 years. Subjects' characteristics are shown in **Table 1**.

The mean body weight and height of STH-infected subjects were lower than those of uninfected subjects, although statistically insignificant with P values of 0.85 and 0.37 respectively. Meanwhile mean BMI was higher in STH-infected than uninfected subjects, although it is also statistically insignifant (P=0.64) (**Table 1**). Forty (31.7%) students were infected with at least one type of intestinal parasite. Thirty (75%) infected subjects were positive for Trichuris trichuria infection only; 7 (17.5%) subjects were positive for Ascaris lumbricoides only; and 3 (7.5%) subjects were positive for mixed infection with both species. Infected students were distributed roughly equally in every age group (**Figure 1**) and

Table 1. Characteristics of subjects

Characteristics	STH infected (n=40)	Uninfected (n=86)	All subjects (N=126)
Mean age (SD), years	9.98 (2.22)	10.15 (1.77)	10.1 (1.9)
Gender, n(%)			
Male	26 (38.81)	41 (61.19)	67 (100)
Female	14 (23.73)	45 (76.27)	59 (100)
Mean body weight (SD), kg	25.89 (7.44)	26.15 (7.03)	26.07 (7.13)
Mean body height (SD), m	129.40 (11.16)	131.08 (9.06)	130.55 (9.76)
Mean BMI (SD), kg/m^2	15.19 (2.29)	14.97 (2.53)	15.04 (2.45)
Infection types, n (%)			
T. trichuria only	30 (75)	-	30 (75)
A. lumbricoides only	7 (17.5)	-	7 (17.5)
Mixed	3 (7.5)	-	3 (7.5)

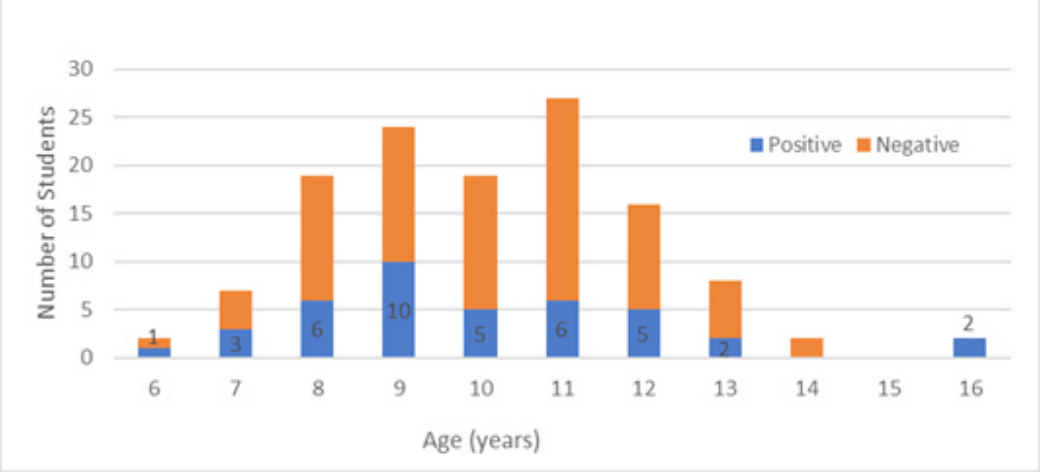

Figure 1. Age distribution of infected students

more boys than girls had STH infection. However, no significant differences were found with regards to age and sex between the infected and uninfected students.

Possible risk factors for STH infection were also analyzed. Chi-square analysis revealed significant relationships between the prevalence of intestinal parasite infection and the following variables: not

Table 2. Suspected risk factors for STH infection

Variables	STH-infected (n=40)	Uninfected (n=86)	P value	Crude odds ratio (95%CI)
Gender				
Female	14 (23.7)	45 (76.3)		1
Male	26 (38.8)	41 (61.2)	0.085	2.03 (0.94 to 4.43)
Hand washing before meals				
Yes	40 (31.7)	86 (68.3)		-
No	0	0		-
Handwashing method				
Water+soap	38 (31.1)	84 (68.9)		1
Water only	2 (50)	2 (50)	0.59	2.21 (0.3 to 16.29)
Handwashing after defecation				
Yes	37 (30.6)	84 (69.4)		1
No	3 (60)	2 (40)	0.33	3.41 (0.55 to 21.23)
Handwashing method after defecation				
Water+soap	34 (29.8)	80 (70.2)		1
Water only	6 (50)	6 (50)	0.19	2.35 (0.71 to 7.82)
Playing with or on soil frequently				
No	23 (32.4)	48 (67.6)		1
Yes	17 (30.9)	38 (69.1)	1	0.93 (0.44 to 1.99)
Eating while playing with soil or objects covered with soil particles (n=55)*				
No	16 (32)	34 (68)		1
Yes	1 (20)	4 (80)	1	0.53 (0.06 to 5.14)
Handwashing after playing with soil or objects with soil particles (n=55)*				
Yes	17 (30.9)	38 (69.1)		1
No	0	0		-
Always using footwear outdoors**				
Yes	34 (29.1)	83 (70.9)		1
No	6 (66.7)	3 (33.3)	0.03	4.88 (1.15 to 20.65)
Playing outdoors without footwear				
Yes	27 (36)	48 (64)		1
No	13 (25.5)	38 (74.5)	0.25	0.61 (0.28 to 1.37)
Cutting nails once per week**				
Yes	32 (28.6)	80 (71.4)		1
No	8 (57.1)	6 (42.9)	0.05	3.33 (1.07 to 10.37)
Nail-biting behavior				
No	33 (32)	70 (68)		1
Yes	7 (30.4)	16 (69.6)	1	0.93 (0.35 to 2.47)
Availability of proper toilet at home**				
Yes	18 (21.2)	67 (78.8)		1
No	22 (53.7)	19 (46.3)	0.0001	4.31 (1.93 to 9.64)
History of anti-helminthic drug consumption within the 2 weeks prior to the study				
Yes	38 (30.9)	85 (69.1)		1
No	2 (66.7)	1 (33.3)	0.24	4.47 (0.39 to 50.85)

* Only 55 students gave answers to "Playing with soil or on soil frequently"
** Significant result was found

using footwear outdoors, no weekly habit of cutting nails, and lack of an available and proper toilet at home (**Table 2**). Children who did not use footwear outdoors [OR 4.88; 95%CI 1.15 to 20.65] and/or did not routinely cut their nails weekly [OR 3.33; 95%CI 1.07 to 10.37] had a higher chance of STH infection than children who used footwear outdoors and cut their nails weekly. The strongest association with STH infection was the lack of an available and proper toilet at home [OR 4.31; 95%CI 1.93 to 9.54]. Further multivariate analysis revealed that only the last variable was significantly associated with STH infection [adjusted OR = 3.94; 95%CI 1.72 to 8.98].

Assessment of individuals' weight-, height-, and BMI-for-age were compared to the WHO population growth chart (**Table 3**).[10] We found that 39.4% of subjects less than 10 years old were underweight; 25.4% of all subjects were stunted; and 30.2% fell within the range of thin to severely thin. Bivariate analysis of STH infection status and each nutritional parameter revealed no significant associations (**Table 4**).

Table 3. Nutritional parameters of the subjects

Parameters	
Weight-for-age category based on WHO Z-score, n (%)	(N=71)*
Severely underweight (<-3 SD)	9 (12.7)
Underweight (-3 SD to < -2SD)	19 (26.8)
Normal weight for age (≥-2 SD)	43 (60.6)
Height-for-age category based on WHO Z-score, n (%)	(N=126)
Severely stunted (<-3 SD)	6 (4.8)
Stunted (-3 SD to < -2SD)	26 (20.6)
Normal height for age (≥ -2 SD)	94 (74.6)
BMI-for-age category based on WHO Z-score, n (%)	(N=126)
Severe thinness (<-3 SD)	15 (11.9)
Thinness (≥ -3 SD to < -2SD)	23 (18.3)
Normal BMI for age (≥ -2 SD to ≤ +1 SD)	79 (62.7)
Overweight (> +1 SD to ≤ +2 SD)	6 (4.8)
Obese (> +2 SD)	3 (2.4)

*n=71 (WHO weight-for-age reference data not available beyond age 10)

Table 4. Comparison of nutritional parameters in STH-infected and uninfected subjects

Parameter	STH-infected	Uninfected	P value
Weight-for-age*, n (%)	(n=25)	(n=46)	
< -2 SD	8 (11.3)	20 (28.2)	
≥ -2 SD	17 (23.9)	26 (36.6)	0.448
Heigh-for-age, n(%)	(n=40)	(n=86)	
< -2 SD	12 (9.5)	20 (15.9)	
≥ -2 SD	28 (22.2)	66 (52.4)	0.510
BMI-for-age, n(%)	(n=40)	(n=86)	
< -2 SD	11 (8.7)	27 (21.4)	
≥ -2 SD	29 (23)	59 (46.8)	0.835

*n=71 (WHO weight-for-age reference data not available beyond age 10)

Discussion

Children are the most vulnerable population group for parasitic infections, due to their low level of immunity, frequent contact with soil and other potentially contaminated materials, and lack of understanding the importance of hygiene and health standards.[11] In our study, the overall prevalence of STH infection in school-aged children was relatively high at 31.7%. This result was higher than the 25.7% reported by Siregar,[12] but lower than the 38.57% prevalence in Baturiti, Tabanan.[13] However, a study in Telaga village, near Gegelang, reported that 68.41% of SDN 1 Telaga students and 83.87% SDN 2 Telaga students were infected with Ascaris lumbricoides as the predominant agent.[14] Differences in prevalence can be caused by several factors, such as time of research, geographical location, as well as cultural, social, or economic reasons.[11]

Personal hygiene consisting of hand-washing and outdoor play habits are known to be significant factors on the incidence of STH.[15,16] Another risk factor associated with helminth infection is nail hygiene, because the nails can mediate the entry of worm eggs into the human body. The fingernails can hide soil that contains microorganisms or eggs, and are often difficult to clean.[17] However, by interview, most subjects reported good handwashing practice, routine nail trimming, and outdoor footwear usage. Since not cutting nails once per week was significantly associated with helminth infection, we should emphasize the importance of nail hygiene by periodic trimming.

Playing on the ground is also a risk factor for STH infection, since soil contaminated with feces can be a source of intestinal helminth infections.[18] Samad reported a relationship between soil contamination by *Ascaris lumbricoides* eggs and infection in elementary school children in the Tembung subdistrict of Medan.[19] Also, Sumanto found that the habit of playing on the ground increased the odds of STH infection (OR 5.2).[20] In our study, no significant association was observed between the habit of playing with/on soil environment and STH infection. But we did find a significant difference between groups with regards to footwear usage. Similarly, Maryanti (2006) found that not using footwear was associated with STH infection (OR 8.8), but Sumanto (2010) found no significant results.[20,21]

Improper sanitation is the dominant risk factor for STH infection.[22] We noted that 55% of infected subjects had no proper toilet at home (*vs.* 22% of uninfected subjects), and it was the only significant factor in the multivariate analysis. A previous study found that children who defecated in toilets had a lower prevalence of STH infection compared to children who defecated outdoors, due to soil/environmental pollution by feces containing worm eggs.[21] In our study, more than half of infected subjects did not have toilet at home. This factor was the most significant one related to STH infection. People who lack a proper toilet habitually defecate on the open ground or in the river. As such, recurrent infection can occur as helminth eggs are deposited in the soil, where they develop before becoming infectious and continue their life cycle.[23]

This study was borne out of a suspicion that the mass eradication program was ineffective, since almost all of the children had received drugs in recent weeks before the study. While the *WHO* recommends mass eradication by albendazole (400 mg) or mebendazole (500 mg) as effective, inexpensive, and easy to administer by non-medical personnel (e.g., teachers), a high prevalence of STH infection persists, particularly for *T. trichuria*. Similarly, a Honduras study reported this problem.[24] Possible reasons for this finding are that the helminths were drug-resistant or that patients received an inadequate dose of anti-helminthic drugs. The predominance of *T. trichuria* as the infectious helminth in our study may related to the fact that a single dose of albendazole used in the mass drug administration program is not adequate for eradication. In fact, the efficacy of short course cure rates with albendazole for *T. trichuria* was only 28%, which is fairly low compared to 88% for Ascaris.[25,26] Even the WHO recently recommended using 400 mg albendazole for 3-7 days for *T. trichuria* treatment.[27,28] However, this hypothesis needs further study specifically designed for a larger number of subjects.

In terms of nutritional status, a considerable proportion of our subjects were within less than normal WHO reference values for growth and nutrition. This trend is common for the Indonesian population, albeit, the values were slightly lower than average compared to those reported in the 2013 Indonesian Health Survey (*Riskesdas*).[29] The proportion of children suffering chronic undernutrition (as measured by stunting) was 25.4% in our study. However, the proportion of general undernutrition (as measured by thinness) was 30.2% in our subjects. Both fall roughly within corresponding data from the average population, with exception to weight-for-age, since no published data was available. As mentioned, we aimed to discern potential associations between STH infection and subjects' nutritional status (stunting, underweight, and thinness), but we observed no such associations. Therefore, STH infection was not associated with increased odds of stunting, thinness, or underweight in this study.

Our study had several limitations. Due to the cross-sectional design, we could not determine causal relationships between observed risk factors and STH infection. The direct smear method also may not detect mild infections.[30] Moreover, since only a single stool specimen was collected from each student, there might be significant underestimation of the prevalence of STH because of the temporal variation in egg excretion over hours and days.[31] Consequently, the underestimation may not truly reflect a lack of association between STH infection and nutritional status.

In conclusion, we find a high prevalence of intestinal parasite infection in a school within an area following a mass eradication program. Furthermore, absence of a toilet, no routine nail trimming, and no outdoor footwear usage are found to be associated with STH infection. However, there is no association between STH infection and nutritional status. As a

recommendation, regular STH monitoring, sanitary improvement, and de-worming programs are necessary to reduce the load of infection and effectively minimize eggs being deposited into the environment. These steps may subsequently decrease the rate of recurrent infections. Future study involving a larger sample size and more schools is warranted.

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Conflict of Interest

None declared.

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Original Article

Initial clinical and laboratory profiles to predict pediatric dengue infection severity

Natharina Yolanda, Harris Alfan

Abstract

Background Prior to the critical phase of dengue infection, it is difficult to differentiate between mild and severe dengue. Identifying risk factors for severe dengue from patients' initial presentation would help decrease the need for hospitalization, increase physician awareness, and improve outcomes.

Objective To predict the severity of pediatric dengue infection based on initial patient characteristics as well as routine clinical and laboratory profiles.

Methods This cross-sectional study was based on medical records of children with dengue infection in Atma Jaya Hospital, Jakarta. Inclusion criteria were children aged 1-18 years with proven dengue infection and hospitalized in Atma Jaya Hospital during the study period (January to December 2016). Clinical profiles and laboratory parameters at the time of patient presentation were extracted and analyzed for possible relationships with dengue severity.

Results Data was collected from 110 patients with a mean age of 9.5 (SD 5) years. Initial clinical profiles that were significantly associated with severe dengue were: age ≤5 years (OR 0.113; 95%CI 0,025 to 0.510), hepatomegaly (OR 2.643; 95%CI 1.051 to 6.650), pleural effusion (OR 9.545; 95%CI 3.722 to 24.777), platelet ≤125,000/uL (OR 0.201; 95%CI 0.044 to 0.924), hyponatremia (OR 10.139; 95%CI 2.576 to 39.906), and AST >135 units/L (OR 5.112; 95%CI 1.564 to 15.710). Biological sex, duration of fever, additional symptoms, spontaneous bleeding, blood pressure, pulse pressure, hematocrit, leukocyte count, random blood glucose, calcium, and ALT level had no significant association with dengue severity.

Conclusion Physicians should be conservative in the management of pediatric dengue patients older than 5 years of age, presenting with hepatomegaly, pleural effusion, platelets >125,000/μL, hyponatremia, or AST more than three times the upper limits of normal. These patients have a higher risk of severe dengue than patients without those findings. **[Paediatr Indones. 2017;57:303-9 ; doi: http://dx.doi.org/10.14238/pi57.6.2017.303-9]**.

Keywords: *dengue; severity; children; clinical profiles; prognosisschool-aged children*

Dengue infection may present a broad range of clinical severity, from asymptomatic, dengue fever (DF), dengue hemorrhagic fever (DHF), and finally, dengue shock syndrome (DSS).[1] Prior to the critical phase, it is difficult to differentiate between mild and severe dengue. To date, there are no diagnostic or prognostic tools available to distinguish severe from non-severe dengue, or other febrile illnesses. Despite the recommendation to hospitalize only patients with severe dengue, many patients with suspected dengue are hospitalized for close monitoring.[2] Several clinical profiles and laboratory measures have been studied with regards to predicting dengue

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severity. Among them are sex, age, abdominal pain, hepatomegaly, abnormal bleeding, ascites, pleural effusion, leukopenia, thrombocytopenia, hemoconcentration, and elevated liver enzymes.[3] Most previous studies did not mention the timing of clinical profiles and laboratory parameters in the disease history. Therefore, we intended to explore clinical and laboratory parameters at the time of patient presentation as potential predictors for severe dengue. By identifying these risk factors at the initial patient presentation, we hope to decrease the need for hospitalization, increase physician awareness of the possibility of severe dengue, and improve outcomes of severe dengue by administering earlier treatment.

Methods

This cross-sectional study was based on medical records of pediatric patients in Atma Jaya Hospital, Jakarta. Inclusion criteria were inpatient pediatric patients aged 1-18 years with a final diagnosis of dengue infection, from January to December 2016. The data were retrieved from hospital medical records with the following ICD-10 codes: A90-dengue fever, A91-dengue hemorrhagic fever, and A910-dengue hemorrhagic fever with shock. Diagnosis of dengue infection was based on criteria mentioned in **Table 1** and confirmed by serologic parameters, i.e., NS1 or anti-dengue IgM. Exclusion criteria were signs of shock at the time of presentation, unproven dengue infection, and comorbidities with other illnesses.

Table 1. Dengue infection diagnosis criteria[4]

1. Dengue infection (DF): acute or abrupt onset of fever, accompanied by a positive tourniquet test, and white blood count ≤ 5,000/µL;
2. Dengue hemorrhagic fever (DHF)
   All of the following items:
   (i) Acute or abrupt fever for 2–7 days,
   (ii) At least one of the following bleeding episodes:
      a. positive tourniquet test,
      b. petechiae, ecchymoses, or purpura,
      c. bleeding from mucosa, gastrointestinal tract, injection sites, or other locations,
      d. hematemesis or melena,
   (iii) Platelet count ≤ 100,000/µL,
   (iv) At least one of the following evidences of plasma leakage:
      a. hemoconcentration assessed by an increase in hematocrit ≥ 20% from previous hematocrit,
      b. signs of plasma leakage, such as pleural effusion or ascites, or evidence of hypoalbuminemia;
3. Dengue shock syndrome (DSS): all items for dengue hemorrhagic fever above, accompanied by evidence of circulatory failure:
   (i) pulse pressure ≤ 20mmHg,
   (ii) or manifested by hypotension, cold body temperature or irritability.

Table 2. Dengue severity

Dengue severity was classified into 4 grades, based on bleeding episodes and shock, as follows:
Grade 1: no evidence of bleeding, positive tourniquet test
Grade 2: evidence of bleeding episodes
Grade 3: presence of weak and rapid pulse rate, low blood pressure, or narrow pulse pressure
Grade 4: non-measurable blood pressure or non-palpable pulse
Grades 1-2 were classified as DHF and grades 3-4 were classified as DSS.

Clinical profiles at the time of patient presentation in the emergency room or outpatient clinic were extracted from medical records and tested as variables. Clinical profiles at the time of presentation were: (1) age and sex, (2) duration of fever at presentation, (3) signs and symptoms, (4) hemodynamic parameters (systolic and diastolic blood pressure, pulse pressure), (5) routine laboratory measures (hematocrit, leukocyte, and platelet counts), (6) random blood glucose, (7) electrolytes (sodium and calcium), (8) and liver function tests [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)]. Laboratory parameters used were the first laboratory data at the time of presentation, before starting intravenous fluid.

Age was categorized as ≤ 5 years (toddler) and > 5 years (school age), as it has been reported that extremes of age are related to dengue severity.[5] Cut-off values for systolic, diastolic, and mean arterial blood pressures were based on minimum values in children aged > 1 year. We used a hematocrit cut-off of 50%, as set by the WHO as the warning value. For platelet analysis, we set the cut-off value at 125,000/µL, a little lower than the minimum normal value to achieve high predictive sensitivity. An AST cut-off point of 135 units/L was used, as it was three-times the normal upper limit and other studies noted mean liver enzyme values in dengue patients to be 2-3 times the normal value. For analysis, dengue cases were categorized as mild dengue (dengue fever, grades I and II DHF) and severe dengue (grades III and IV DHF) (**Table 2**).

All data were analyzed using the *Statistical Packages for Social Sciences (SPSS) software version 22.0*. Potential predictors mentioned above were tested against the categories of dengue cases using Chi-square or Fisher's exact test, as appropriate. The predictive ability was presented with odds ratio. P values <0.05 were considered to be statistically significant. This study was approved by the Ethics Committee of Atma Jaya Hospital, Jakarta.

Results

One hundred ten subjects' medical records were analyzed. Most of the cases were dengue fever (51%), followed by DHF grade III (16%), DHF grade IV (15%), DHF grade I (10%), and DHF grade II (8%). There were 56% male and 44% female subjects, with mean age 9.5 (SD 5) years. The most common symptoms at presentation were vomiting (83%), abdominal pain (66%), and headache (55%). Myalgia, pleural effusion, hepatomegaly, diarrhea, and cough were found to lesser extents. The mean values of hemodynamic parameter and laboratory tests are presented in **Table 3**. Data on sodium, calcium, blood glucose, AST, and ALT were incomplete; the missing data were replaced by the mean value of each corresponding group.

Bivariate analysis revealed that the clinical profiles significantly related to severe dengue were age ≤5 years (OR 0.113; 95%CI 0.025 to 0.510; P=0.001), hepatomegaly (OR 2.643; 95%CI 1.051 to 6.650; P=0.035), pleural effusion (OR 9.545; 95%CI 3.722 to 24.777; P=0.000), platelets ≤125,000/uL (OR 0.201; 95%CI 0.044 to 0.92; P=0.025), hyponatremia ≤128 mmol/L (OR 10.139; 95%CI 2.576 to 39.906; P=0.000), and AST >135 units/L (OR 5.112; 95%CI 1.564 to 15.710; P=0.004). Initial data on biological sex, duration of fever, symptoms, bleeding manifestation, hemodynamic parameter, hematocrit, leukocyte, random blood glucose, calcium, and ALT were not significantly related to severe dengue (**Table 4**).

Table 3. Characteristics of subjects

Characteristics	Value
Sex, n(%)	
Male	62 (56)
Female	48 (44)
Mean age (SD), years	9.5 (5)
Mode of presentation, n(%)	
Headache	61 (55)
Myalgia	34 (30)
Vomiting	91(83)
Cough	7 (6)
Abdominal pain	73 (66)
Diarrhea	25 (23)
Hepatomegaly	25 (23)
Pleural effusion	32 (29)
Petechiae	30 (27)
Any bleeding manifestation	17 (15)
Hemodynamics	
Mean SBP (SD), mmHg	102 (10
Mean DBP (SD), mmHg	677 (13)
Mean pulse pressure (SD), mmHg	79 (11)
Laboratory findings	
Mean hematocrit (SD), %	40 (5)
Mean leucocytes (SD), /µL	4,600 (3,400)
Mean platelet (SD), /µL	87,000 (53,000)
Mean random blood glucose (SD), g/dL	107 (19)
Electrolytes	
Mean sodium (SD), mEq/L	127 (2.5)
Mean calcium (SD), mEq/L	1.0 (0.3)
Liver function	
Mean ALT (SD), unit/L	134 (125)
Mean AST (SD), unit/L	54 (51)
Diagnosis, n(%)	
DF	56 (51)
DHF I	11 (10)
DHF II	9 (8)
DHF III	18 (16)
DHF IV	16 (15)

Discussion

Predicting dengue severity based on clinical profiles at the initial time of patient presentation at the health facility is a potential way to reduce morbidity, mortality, and hospital costs. Although most dengue cases do not progress to severe disease, a small percentage of cases that do so are often not predicted initially. Dengue patients in the early course of infection often present to the emergency department or as outpatients in a generally healthy condition, obscuring the potential for severe dengue. Potts *et al.* showed that early clinical indicators could be used to differentiate between mild and severe dengue before plasma leakage occurred.[2]

Table 4. Bivariate analysis between initial clinical data and dengue severity

	Mild dengue (n=76)	Severe dengue (n=34)	Total (N=110)	OR	95%CI	P value (2 sided)
Sex, n(%)						
Male	33 (69)	15 (31)	48	0.972	0.430 to 2.196	0.946
Female	43 (69)	19 (31)	62			Ref
Age, n (%)					0.025 to 0.510	0.001
≤ 5 years old	49 (60)	32 (40)	81	0.113		Ref
> 5 years old	27 (93)	2 (7)	29			
Fever						
≤3 days	20 (65)	11 (35)	31	0.747	0.309 to 1.803	0.515
>3 days	56 (71)	23 (29)	79			Ref
Headache, n(%)	41 (67)	20 (33)	61	1.220	0.538 to 2.765	0.634
Myalgia, n(%)	21 (62)	13 (38)	34	1.621	0.689 to 3.813	0.266
Vomiting, n(%)	60 (66)	31 (34)	91	2.756	0.746 to 10.183	0.117
Cough, n(%)	6 (86)	1 (14)	7	0.354	0.041 to 3.057	0.325
Abdominal pain, n(%)	50 (68)	23 (32)	73	1.087	0.460 to 2.571	0.849
Diarrhea, n(%)	17 (68)	8 (32)	25	1.068	0.409 to 2.785	0.893
Hepatomegaly, n(%)	13 (52)	12 (48)	25	2.643	1.051 to 6.650	0.035
Pleural effusions, n(%)	11 (34)	21 (66)	32	9.545	3.722 to 24.777	0.000
Petechiae, n(%)	18 (60)	12 (12)	30	1.758	0.729 to 4.237	0.206
Any bleeding manifestation, n(%)	13 (76)	4 (24)	17	0.646	0.194 to 2.150	0.474
Hemodynamic						
SBP, n (%)						
≤ 90 mmHg	18 (78)	5 (22)	23	1.800	0.607 to 5.335	0.285
> 90 mmHg	58 (67)	29 (33)	87			Ref
DBP, n (%)						
≤ 60 mmHg	35 (73)	13 (27)	48	1.379	0.604 to 3.149	0.445
> 60 mmHg	41 (66)	21 (34)				Ref
Pulse pressure						
≤ 65 mmHg	3 (60)	2 (40)	5	0.658	0.105 to 4.127	0.644
> 65 mmHg	73 (70)	32 (30)	105			Ref
Laboratory						
Hematocrit, n (%)						
> 50%	2 (33)	4 (67)	6	4.933	0.858 to 28.378	0.051
≤ 50%	74 (71)	30 (29)	104			Ref
Leukocyte, n (%)						
≤ 5,000/µL	53 (68)	25 (32)	78	0.830	0.335 to 2.052	0.686
> 5,000/µL	23 (72)	9 (28)	25			Ref
Platelet, n (%)						
≤ 125,000/µL	58 (64)	32 (36)	90	0.201	0.044 to 0.924	0.025
> 125,000/µL	18 (90)	2 (10)	20			Ref
Random blood glucose, n (%)						
≤ 100 mg/dL	18 (69)	8 (31)	26	1.009	0.389 to 2.615	0.986
> 100 mg/dL	58 (69)	26 (31)	34			Ref
Electrolyte						
Sodium, n (%)						
≤ 128 mmol/L	73 (75)	24 (25)	97	10.139	2.576 to 39.906	0.000
> 128 mmol/L	3 (23)	10 (77)	13			Ref
Calcium, n (%)						
≤ 1 mmol/L	70 (78)	20 (22)	90	7.000	0.603 to 81.228	0.138
> 1 mmol/L	1 (33)	2 (67)	3			Ref

to be continued

Table 4. Bivariate analysis between initial clinical data and dengue severity (continued)

Liver function test						
AST, n (%)						
> 135 units/L	5 (36)	9 (64)	14	5.112	1.564 to 15.710	0.004
≤ 135 units/L	71 (74)	25 (26)	96			Ref
ALT, n (%)						
> 135 units/L	2 (40)	3 (60)	5	3.581	0.570 to 22.493	0.170
≤ 135 units/L	74 (70)	31 (30)	105			Ref

SBP: systolic blood pressure; DBP: diastolic blood pressure; AST: aspartate transaminase; ALT: alanine transaminase

The WHO stated the warning signs of dengue: abdominal tenderness, hepatomegaly, lethargy, cold extremities, bleeding, platelet ≤ 75,000/mm^3, and hematocrit value of 50%, or a rise of more than 22% from baseline hematocrit. Initially mild dengue cases may later develop into severe dengue without any warning signs.[1] This fact warrants the search for other factors to help early prediction. Other risk factors were Caucasian race, people with AB blood group, and, extreme ages, and coexisting conditions.[1]

Several prognostic tools for dengue have been developed, e.g., the decision tree algorithm,[2] the diagnostic dengue infection severity score,[3] decision algorithms,[6] the pediatric logistic organ dysfunction score,[7] and the disseminated intravascular scoring system.[8] Previously, a dengue infection severity score developed in Thailand included age, hepatomegaly, systolic blood pressure, white cell count, and platelets as significant predictors and scoring items. Pongpan *et al*. used the scoring system to correctly classify patients into their original severity levels of DF, DHF, or DSS, with clinically acceptable over- and underestimation.[3,9]

We found age, hepatomegaly, pleural effusion, platelets > 125,000/μL, hyponatremia ≤128 mmol/L, and AST >135 units/L to be significant predictors for severe dengue. Some epidemiological studies reported that the two extremes of age (young and old) were associated with severe dengue.[1,10] This association was noted in WHO recommendations, in which infants and the elderly are included as indications for hospital referral.[11] with regards to age as a risk factor for severe dengue, Lovera et al. noted higher risk of severe dengue in children aged >5 years,[12] but Martina *et al.* reported higher risk in children <5 years old.[13] Different populations, study designs, and analyses in those studies might have affected the results. Young age was hypothesized to be related to high microvascular fragility and low vascular adaptation capacity, leading to higher risk of shock. However, in older children, i.e., > 5 years old, reinfection with different serotypes often lead to severe dengue (antibody-dependent enhancement).[13]

Hepatomegaly is a well-known physical finding in dengue. Moderate liver enlargement is a normal response to dengue infection. Several studies found a correlation between hepatomegaly, increased ALT, and dengue severity.[14,15] Clinically, hepatomegaly is one of the causes of abdominal pain, which is included in the WHO warning signs. In our study, risk of developing shock was two times higher in patients with hepatomegaly than those without hepatomegaly. A previous meta-analysis found the risk to be up to five times higher.[16] However, it should be kept in mind that physical examination to determine hepatomegaly in children is operator-dependent and requires appropriate skill.

Yacoub et al. noted that clinical plasma leakage, i.e., pleural effusion, ascites, and gall bladder wall edema were correlated with disease severity.[10] Even though gall bladder wall edema was known to precede the development of ascites and effusion, abdominal ultrasonography to detect such findings was not routinely performed in the febrile phase.

Thrombocytopenia is an important laboratory finding and one of the diagnostic criteria for dengue infection. Severity of thrombocytopenia was correlated with plasma viral load and the extent of plasma leakage. Several previous studies noted different cut-off platelet counts to predict the development of shock.[12,13] We found a significant correlation with development of severe dengue using a platelet count cut-off of ≤ 125,000/μL. This cut-off was not as low as in previous studies,[3,17] and, moreover, did not fulfill the minimum WHO diagnostic criteria (i.e., < 100,000/μL), but it could be considered as a sensitive predictor for severe dengue.

Hyponatremia and high AST showed the highest

odds ratio (approximately 10 and 5, respectively), but analysis of those two variables used mean value to fill the missing data. We preferred to consider hyponatremia and AST >135 units/L as potential predictors for severe dengue, but further studies are needed. Based on several previous studies, hyponatremia was the most common electrolyte disturbance in dengue (61% of DF patients and 72% of DHF patients). Low sodium levels are related to complications, such as central nervous system disturbance and bleeding.[18,19] The AST elevation was usually higher than ALT, probably due to involvement of myocytes in dengue patients. Several studies stated that AST was associated with severity of infection, but they did not discriminate between non-severe and severe dengue.[20,21] Liver enzymes are known to peak late in the course of dengue (day 6-7), therefore, their usefulness as prognostic markers was limited.[10]

Hematocrit is in the WHO diagnostic criteria. Increased hematocrit is one of the warning signs for progression to shock. Our analysis with a 50% cut-off value had a nearly significant P value; if the sample size had been larger, the P value may have been significant, indicating that hematocrit level >50% at initial presentation could be a predictive parameter for dengue progression to shock. Hematocrit level is a common method to identify and monitor plasma leakage. However, this method might be rather insensitive (particularly if patients had received parenteral fluid) and limited by the fact that an individual's baseline value was usually not known.[10]

Hemodynamic parameters were found to not be predictive of severe dengue. However, the parameters used in the analysis were the findings at the initial patient presentation (which mostly presented in the fever stage) and any patients with hemodynamic disturbance were excluded as subjects. As such, abnormalities in hemodynamic parameters were one of the severe dengue criteria (dengue shock syndrome), therefore, it could not be used as predictor.

There were several limitations in our study. This study was retrospective in design and based on medical records, with potential bias of incomplete documentation and variations in skills of examining physicians. We also did not take other potential parameters into account, such as laboratory bleeding parameters and other hepatic function tests. Despite the limitations, our findings suggested several risk factors that may be useful for predicting severe dengue early at the time of patient presentation. Further studies with a larger number of subjects and a randomized, prospective design are needed.

In conclusion, initial clinical findings related to impending severe dengue were age older than 5 years old, hepatomegaly, pleural effusion, platelets > 125,000/μL, hyponatremia, and AST more than three times the upper normal limit. Physicians need to be cautious if dengue patients present with one or more of these findings.

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Conflict of Interest

None declared.

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Evidence-based Case Report

Side effect of deferiprone as iron chelator in children with thalassemia

Mikhael Yosia, Pustika Amalia Wahidiyat

Abstract

Background There are currently three available iron chelators: deferoxamine (DFO), deferasirox (DFX), and deferiprone (DFP). In Dr. Cipto Mangunkusumo Hospital and Indonesia, in general, the accessibility of DFP for thalassemia patients has been adequate. Even though its efficacy in removing iron has been proven by countless studies, questions relating to its safety and possible side effects continue to be raised.

Objective To assess common side effects of DFP usage by an intensive literature search and compare them to that in a pediatric thalassemia patient, in order to determine if the child's symptoms were potentially caused by DFP.

Methods A literature search using MeSH terms was done in PubMed. Full copies of articles that fulfil the inclusion criteria, based on their title, abstract, and subject descriptors, were critically appraised using The Joanna Brigs Institute (JBI) critical appraisal tools.

Results A total of 10 original articles from 1998-2013 were deemed applicable to this study including: 2 case reports, 5 prospective cohort studies, 2 retrospective cohort studies, and 1 randomized control trial with a grand total of 1,026 subjects.

Conclusion Side effects of DFP include neutropenia, agranulocytosis, increased ALT, gastrointestinal problems, arthralgia or arthropathy, increased appetite or weight, thrombocytopenia, urine discoloration, as well as auditory and visual disturbances. Our case report patient's symptoms of gum bleeding and haemorrhagic mass are not related to her DFP consumption. **[Paediatr Indones. 2017;57:329-36 ; doi: http://dx.doi.org/10.14238/pi57.6.2017.329-36]**.

Keywords: *thalassemia; deferiprone; side effect*

Thalassemia is a condition involving reduction in globin chain (-a or -b) production thus resulting in abnormal hemoglobin which leads to anemia. Anemia often needs to be controlled via continuous blood transfusion. This transfusion, coupled with hemolysis of abnormal hemoglobin and increased rate of iron absorption, results in the build-up of iron in the body. If left untreated, the excessive iron level may harm vital organs (liver, heart, and endocrine organs) and manifest as complications of thalassemia. Thus, iron chelators were introduced as drugs capable of bonding with iron, creating an iron-chelator complex which can then be excreted from the body, ultimately reducing the patient's iron load.[1]

There are currently three available iron chelators: deferoxamine (DFO), deferasirox (DFX), and deferiprone (DFP), each with their own benefits

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and drawbacks. Deferoxamine was the first and most studied iron chelator worldwide, but due to its subcutaneous or intravenous mode of delivery, it has become unpopular with patients. The DFP and DFX are available in oral form, which eases usage and increases adherence (thus increase the overall survival rate) in thalassemia patients.[2] Differences in molecular size of the chelators result in different iron-chelator interactions. Hence, each chelator has its own effectivity and side effects.

In Dr. Cipto Mangunkusumo Hospital and Indonesia, in general, accessibility to DFP has been adequate. The fact that this particular iron chelator is available in both tablet and syrup form, adds to its popularity among patients. Though its efficacy in removing iron has been established by many studies, 21 questions relating to DFP safety and possible side effects continue to be raised.[3] We aimed to compare a case in a real clinical scenario to the published side effects of DFP from a literature review, and to determine if the patient's symptoms were related to consumption of DFP.

The Case

An 11-year-old girl was diagnosed with beta-thalassemia major at the age of 5. She had routine blood transfusion every 2 weeks and had been prescribed with DFP (100 mg/kg body weight/day) for 4 years. For 3 months, she had gum bleeding, especially when brushing her teeth. Soon after, a hemorrhagic mass developed in her oral cavity and emitted a pungent odor. She was taken to a dental clinic; the dentist administered treatment and drugs, but to no avail. After 2 weeks of treatment, her bleeding episodes had not subsided, so her mother took her to Dr. Cipto Mangunkusumo Hospital, where she was diagnosed with suspected gingival enlargement and oral cavity infection by an oral surgeon. The surgeon recognised that her condition might have been caused by toxicity of DFP. Her blood examinations revealed hemoglobin level of 8.5 g/dL, hematocrite 25.1%, leucocyte 6,490/μL, platelet 184,000/μL, AST 23 U/L, and ALT 10 U/L.

Clinical Questions

1. What were the common side effects of DFP?
2. Were the patient's symptoms related to DFP usage?

Methods

Literature included in this review comprised studies with human subjects of any age with thalassemia, intervention included continuous usage of DFP monotherapy at any dose, outcomes measured were any observed or suspected short-term or long-term side effects or adverse reactions to DFP. All randomized control trials (RCTs), prospective, retrospective, as well as cross-sectional studies, with full text available in English or Indonesian ,which were published within the last 20 years, were included.

The initial search terms used were 'deferiprone', 'side effect', 'safety', and 'thalassemia', followed by proper MeSH search terms in PubMed (**Table 1**). Full copies of articles identified by the search that fulfilled the inclusion criteria, based on their title, abstract, and subject descriptors, were critically appraised using The Joanna Brigs Institute (JBI) critical appraisal tools.[22] Pubmed search was done in 10 April 2017.

Table 1. MeSH search terms in PubMed followed by number of hits and articles selected

Database	Search Terms	Hits	Selected articles
PubMed	("thalassaemia"[All Fields] OR "thalassemia"[MeSH Terms] OR "thalassemia"[All Fields]) AND ("deferiprone"[Supplementary Concept] OR "deferiprone"[All Fields]) AND ("safety"[MeSH Terms] OR "safety"[All Fields]) AND ("adverse effects"[Subheading] OR ("adverse"[All Fields] AND "effects"[All Fields]) OR "adverse effects"[All Fields] OR ("side"[All Fields] AND "effects"[All Fields]) OR "side effects"[All Fields])	70	12

Results

The search procedure is shown in **Figure 1**. A total of 10 original articles from 1998-2013 were included in this study. A summary of characteristics (including total number of subjects with/without side effects) of included literatures is shown in **Table 2**, whilst the list of side effects observed in each study was recorded in **Table 3**. Frequency of each side effect are presented in **Table 4**, with the total frequency from all the studies (total subjects showing side effects n=427) is presented in the last column. Note that only one study reported skin rash and one other study reported auditory and visual disturbances.

A case report by Chand *et al.* reported an 8-year-old arthropathy patient with uncontrollable ferritin level due to poor adherence to DFP. After 1 year of consuming DFP, the patient complained of pain in both knees. The administering doctor stopped the DFP for 3 months and the symptoms subsided.[5] A second case study by Tewari *et al.*[11] reported a possible periodontal manifestation of agranulocytosis caused by DFP. This patient complained of grayish-white discoloration of his gingiva, followed by tooth pain. He had consumed DFP for 3 years and suffered from episodes of neutropenia and agranulocytosis. After four weeks of stopping DFP consumption, the neutropenia subsided and signs of clinical improvement were present. All studies were appraised using the appropriate JBI critical appraisal tools (**Table 5**).

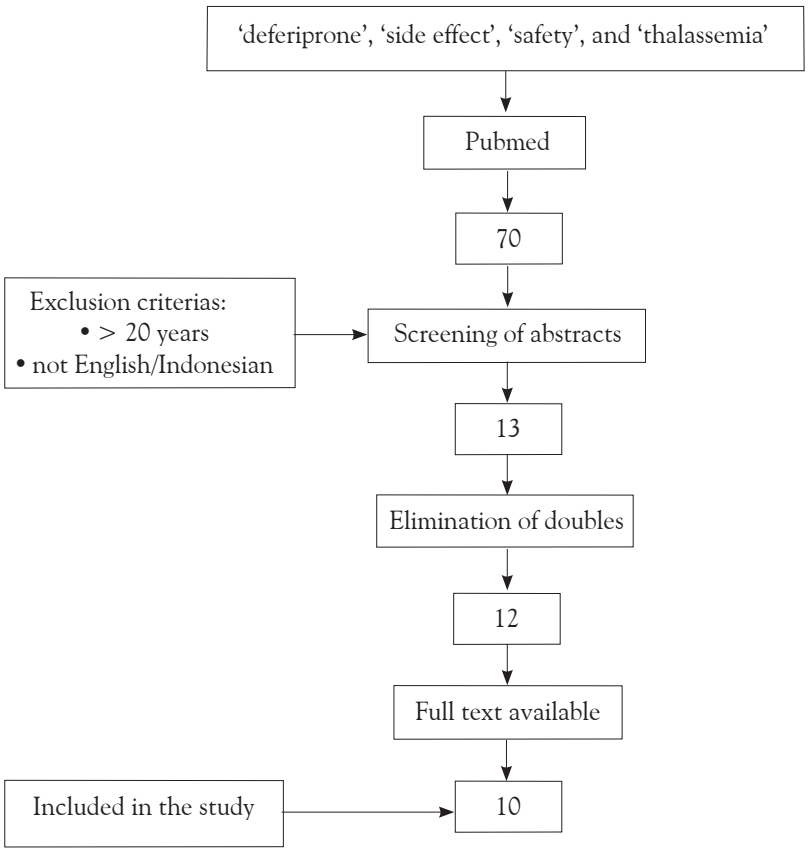

Figure 1. Flow chart of the literature search procedure

Table 2. Summary of study design and characteristics

Design	Year of study	Age range, years	Subjects, n	Length of study, months	Dose range, mg/kg body weight/day	Author
Prospective cohort	2002	6-54	532	36	75	Ceci *et al.*[4]
Case report	2009	8 months[a]	1	-	40-80	Chand *et al.*[5]
Prospective cohort	2004	4-14	75	12	50 and 75	Choudhry *et al.*[6]
Prospective cohort	1998	10-41	187	12	75	Cohen *et al.*[7]
Prospective cohort	2010	1-10	100	6	50-100	El-Alfy *et al.*[18]
Randomized controlled trial	2006	8-40	6	18	75	Ha *et al.*[8]
Prospective cohort	1998	22-38	51	24-48	50-79	Hoffbrand *et al.*[9]
Retrospective cohort	2005	2-6	44	-	75	Naithani *et al.*[10]
Case report	2009	14[a]	1	-	75	Tewari *et al.*[11]
Retrospective cohort	2013	5-52	29	-	26-85	Uygun *et al.*[12]

[a]Case report with one patient used the exact age

Table 3. Summary of reported side effects

Author	Reported side effects
Ceci *et al.*[4]	Neutropenia, agranulocytosis, increased ALT, gastrointestinal problem, arthralgia/arthropathy, increased weight
Chand *et al.*[5]	Arthralgia/arthropathy
Cohen *et al.*[7]	Neutropenia, agranulocytosis, increased ALT, gastrointestinal problem, arthralgia/arthropathy, increased in appetite/weight, thrombocytopenia
El-Alfy *et al.*[18]	Neutropenia, arthralgia/arthropathy, increased ALT, gastrointestinal problem
Ha *et al.*[8]	Arthralgia/arthropathy, increased ALT, gastrointestinal problem
Hoffbrand *et al.*[9]	Neutropenia, agranulocytosis, arthralgia/arthropathy, gastrointestinal problem (nausea)
Naithani *et al.*[10]	Gastrointestinal symptoms, urine discoloration, arthralgia/arthropathy, neutropenia, thrombocytopenia
Tewari *et al.*[11]	Agranulocytosis (manifest as necrotizing stomatitis)
Uygun *et al.*[12]	Gastrointestinal symptoms, neutropenia, increased ALT, auditory disturbance, visual disturbance

ALT: alanine aminotransferase

Discussion

The aim of this study was to compare common side effects of DFP usage found in the literature to symptoms in a pediatric thalassemia patient who consumed DFP. None of the articles mentioned side effects similar to the symptoms presented in the clinical scenario (bleeding gums, followed by a mass growing in the oral cavity). The patient's however did not suffer from any other side effects mentioned in all the literatures. Other examinations need to be done in order to determine the exact cause of symptoms in our thalassemia patient.

Neutropenia and agranulocytosis

Severe neutropenia is defined as absolute neutrophil count (ANC) <500/uL, while agranulocytosis is defined as ANC <100/uL.[13] In the literature review, a total incidence (from literature reporting on neutropenia) of 5.5% was established, which was slightly lower than that on the DFP package insert (6.2%) submitted to the FDA (**Table 6**). Neutropenia after DFP consumption is hard to determine, as there is no known underlying mechanism to explain the event.[4] Maggio *et al*. compared sequential DFO and DFP therapy *vs.* DFP monotherapy and found that no agranulocytosis occurred in those who underwent sequential therapy, while 3.7% of

those who underwent DFP monotherapy developed agranulocytosis. They proposed that the reduced bone marrow exposure to DFP in sequential therapy might reduce the chance of agranulocytosis.[14] Masera et al. reported on a patient with agranulocytosis; when treatment with DFP was stopped and corticosteroids were administered, the neutrophil count increased after one day. They reckoned that an immune mechanism blocked myeloid differentiation during promyelocyte phase.[15] Most of the literature included in this study recommended an immediate switch to another available iron chelator, whenever neutropenia or agranulocytosis occurred due to DFP consumption. Tewari *et al.* reported that agranulocytosis resulted in necrotizing stomatitis, which was manifested by white-grayish discoloration on the palatum with no signs of bleeding or inflammation. No other study reported or explained any mechanism resulting in a periodontal manifestation, such as our patient had.

Table 4. Summary of side effect frequencies for each study

Side effect observed	Frequencies (%)[a]								
	Ceci *et al.*[4]	Choudry *et al.*[6]	Cohen *et al.*[7]	El-alfy *et al.*[18]	Ha *et al.*[8]	Hoffbrand *et al.*[9]	Naithani *et al.*[10]	Uygun *et al.*[12]	Total[b]
Neutropenia	21 (3.9)	12 (16)	10 (5)	6 (6)		2 (3.9)	2 (4.5)	3 910.3)	56 (5.5)
Agranulocytopenia	5 (0.9)					1 (1.9)			6 (1)
Thrombocytopenia			2 (1)				20 (45.4)		22 (9.5)
Increased ALT	15 (2.8)			12 (12)	6 (23)			2 (6.9)	35 (5.2)
Gastrointestinal problem	17 (3.2)		70 (37)	11 (11)	8 (31)	5 (9.8)	12 (27.2)	6 (20.6)	129 (13.6)
Athralgia/ arthropathy	21 (3.9)	15 (20)	12 (6)	4 (4)	4 (15)	5 99.8)	4 (9.1)		65 (6.5)
Increaste in appetite/weight	1 (0.2)		10 (5)						11 (1.5)
Urine discoloration			74 (40)				23 (52.2)		97 (42)
Skin rash					1 (4)				1 (4)
Auditory disturbance								3 (10.3)	3 (10.3)
Visual disturbance								2 (6.8)	2 (6.8)

[a]Frequencies are number of side effect observed, percentage was done based on frequency/total subject in the same study article
[b]For total accumulation of frequencies, percentage was done by dividing the total frequency by the total number of subjects from literatures reporting that side effect.

Table 5. Results of critical appraisal using appropriate JBI appraisal tools

Author	Design	Score based on appropriate JBI appraisal*													Overall appraisal
		1	2	3	4	5	6	7	8	9	10	11	12	13	
Ceci *et al.*[4]	Prospective	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	NA	NA	Included
Chand *et al.*[5]	Case report	Y	Y	Y	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Included
Choudhry *et al.*[6]	Prospective	Y	Y	Y	N	N	Y	Y	Y	Y	Y	Y	NA	NA	Included
Cohen *et al.*[7]	Prospective	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	NA	NA	Included
El-alfy *et al.*[18]	Prospective	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	NA	NA	Included
Ha *et al.*[8]	RCT	Y	Y	Y	N	N	N	Y	Y	Y	Y	Y	Y	Y	Included
Hoffbrand*et al.*[9]	Prospective	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	NA	NA	Included
Naithani *et al.*[10]	Retrospective	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	NA	NA	Included
Tewari *et al.*[11]	Case report	Y	Y	Y	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Included
Uygun *et al.*[12]	Retrospective	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	NA	NA	Included

*Appropriate appraisal for either RCT, cohort (prospective or retrospective), case report study was used.
RCT:13 criteria; cohort:11 criteria.
Y=yes, N=no, U=unclear, NA=not applicable.

Table 6. Side effects of DFP observed in 642 patients (from the DFP package insert)[19]

Body sistem Preferred term	Subjects, %
Blood and lymphatic system disorders	
Neutropenia	6.2
Agranulocytosis	1.7
Gastrointestinal disorders	
Nausea	12.6
Abdominal pain/discomfort	10.4
Vomiting	9.8
Diarrhea	3
Dyspepsia	2
Investigations	
ALT increase	7.5
Neutropenia	7.3
Increased body weight	1.9
Metabolic and nutritional disorders	
Increased appetite	4
Decreased appetite	1.1
Musculoskeletal and connective tissue disorders	
Athralgia	9.8
Back pain	2
Pain in extremities	1.9
Arthropathy	1.4
Nervous system disorders	
Headache	2.5
Urinary disorder	
Chromaturia	14.6

Thrombocytopenia

Naithani *et al.* observed thrombocytopenia in nearly half of their subjects, which subsided after discontinuation of DFP. However, they recommended that extra scrutiny to be taken due to the small sample size.[10] Cohen *et al.* had a larger sample, but found that only 1% of thrombocytopenia incidence was associated with DFP.[7] Other literature had reported various incidence rates, yet many agreed that there was an association between DFP consumption and thrombocytopenia. The mechanism of thrombocytopenia is still unknown.

Increased alanine transaminase (ALT)

Three of the studies mentioned an increase of ALT due to DFP consumption, though incidence varied between 2.8 to 23%.[4,8,12] Cessation of DFP reduced the ALT level when ALT had increased to a very high level, yet often the ALT increase is only temporary and resolves spontaneously. There were concerns that this condition might progress to hepatic cirrhosis, but an ensuing review by Wanless *et al.* found no strong evidence of a correlation.[16]

Gastrointestinal problems

One of the most common complaints in DFP consumers is nausea, vomiting, abdominal pain, and other gastrointestinal problems. The package insert breaks down the list of gastrointestinal problems as nausea (12.6%), abdominal pain (10.4%), vomiting (9.8%), diarrhea (3%), and dyspepsia (2%).[19] The incidence can reach 37% and usually occur during initial consumption of DFP (±1 year).[7] The symptoms are usually mild and spontaneously resolve in the majority of patients. Cessation of DFP might be needed when symptoms persist. Interestingly, El-Alfy *et al.*[18] compared the use of syrup DFP to the usual tablet form. In the end, the difference in incidence of gastrointestinal symptoms between the two forms of DFP was inconclusive, but availability of the syrup form would surely help patients who cannot swallow DFP tablets.

Arthralgia/arthropathy

Another common side effect of DFP is joint pain without swelling, usually affecting the knee. The time when this side effect occurs after consumption

of DFP varies between individuals. Most of the time, termination of DFP is needed until all symptoms subside. While the underlying mechanism is not known, Berkovitch *et al.*[17] postulated that due to low concentration of DFP in synovial fluid, less inert 1:3 iron-chelator complexes are formed, and a subsequent increase in 1:1 and 1:2 iron-chelator complex (which are very damaging in nature) accumulate, eventually resulting in symptoms. A case study elaborated that there was no prominent sign of inflammation (swelling or erythema), except for the pain. MRI results present in DFP-related arthropathy are synovial thickening, articular cartilage thickening, and subchondral bone erosions.[5]

Increased in appetite/weight

Weight gain has been identified as an effect of DFP consumption. Even though two studies included observations on weight increase, there were no details regarding how much weight was gained or whether it was statistically significant. The DFP package insert also affected increased appetite (4%) and weight gain (1.9%). There has not yet been known mechanism that explains these effects.[4,7]

Urine discoloration

Two studies reported that nearly half of their sample had red-brown urine discoloration without accompanying symptoms of dysuria, increase in urinary frequency, or other urinary complaints.[7,10] Urine discoloration itself is the result of the chromophore iron-chelator complex being excreted in the urine. There is no report on harm that might be caused by urine discoloration.

Skin rash

Only Ha *et al.* reported an observation of skin rash and cited DFP as the main culprit.[8] The explanation for the skin rash in the study itself was limited and no other sources reported a similar observation. Though possible, other studies need to be done to determine a cause-effect relationship.

Auditory and visual disturbance

Uygun *et al.*,[12] however, were the only authors reporting both auditory and visual disturbances after DFP consumption. They recommended using a larger sample size to determine the exact effect and relationship of these side effects, whilst recommending a reduction in dosage until the symptoms subside. Another available study mentioned neurotoxicity as causing visual and auditory disturbances in patients using deferoxamine (DFO).[20] Further studies need to be conducted to determine whether similar neurotoxicity is observed in those consuming DFP.

Studies regarding the efficacy, safety, and side effects of using DFP were numerous, yet disparities in incidence rate still occurred, as well as observations of side effects that were previously not reported by other studies. Surely, for the sake of patient safety, more observations with larger sample sizes in longer trial period would be beneficial in determining incidence and previously unknown side effects of DFP usage.

In conclusion, continuous close monitoring of patients who undergo iron chelation therapy is needed to ensure that side effects are treated promptly. Side effects of DFP include neutropenia, agranulocytosis, increased ALT, gastrointestinal problems, arthralgia or arthropathy, increased appetite or weight, thrombocytopenia, urine discoloration, as well as auditory and visual disturbances. There is no evidence relating symptoms in the clinical scenario of our pediatric thalassemia patient to DFP consumption.

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Conflict of Interest

None declared.

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