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Original Article

Electrocardiogram abnormalities in obese adolescents

Elizabeth Joan Salim, Eka Gunawijaya, Ni Putu Veny Kartika Yantie

Abstract

Background Obesity in adolescents is a known risk factor for cardiovascular disease mortality and sudden cardiac death. Obesity is associated with a wide variety of electrocardiogram (ECG) abnormalities.

Objective To assess prevalence and describe the ECG abnormalities in obese adolescents.

Methods This cross-sectional study was conducted at Children's Cardiology Clinic - Integrated Heart Center of Sanglah General Hospital, Denpasar, Bali, by recording ECGs of obese adolescents aged 11-15 years from several junior high schools from December 2016 to April 2017. The inclusion criteria were obese adolescents aged 11 to 15 years, who were willing to participate in the study and provided informed consent.

Results A total of 78 ECGs of obese adolescents (60% male) were selected. Subjects' mean weight and age were 82.6 (SD 15.2) kg and 13.2 (SD 1) years, respectively. Pre-hypertension was found in 25 (32%) subjects, while hypertension was found in 18 (23%) subjects. There were 29 (37%) subjects with abnormal ECGs. Sinus tachycardia was present in 13 (17%) subjects, and sinus arrhythmia was identified in 11 (14%) subjects. Eight (10%) patients experienced prolongation of QTc interval and 5 (6%) patients presented with prolongation of PR interval. There were no shifts of the P wave, QRS wave and T wave axes, changes of P wave morphology, low QRS voltage, T wave flattening, ventricular enlargement, or ST segment changes found in this study.

Conclusion The prevalence of cardiac abnormalities based on ECG examination in obese adolescents is 37%, consisting of heart rhythm abnormalities, prolonged PR interval, and prolonged QTc interval. **[Paediatr Indones. 2020;60:18-23; doi: http://dx.doi.org/10.14238/pi60.1.2020.18-23]**.

Keywords: *electrocardiography; obese; adolescents*

Obesity in adolescents is a community health problem and a factor which increases morbidity and mortality in adolescents.[1] The prevalence of overweight and obesity in adolescents has increased three times during the last two decades in the US, and almost all were classifed as obese by the time they reached adulthood.[2] It is suspected that the increase in obesity prevalence in adolescents is caused by lack of physical activity, changes in lifestyle, and inappropriate nutritional intake. Obesity in adolescents is related to increased risk of type 2 diabetes, hypertension, stroke, metabolic syndrome, and cardiovascular disease.[3]

The National Health Examination Survey in the US estimated that approximately 17% of children and adolescents developed obesity. Another study indicated an increase of obesity prevalence in adolescents ranging from 12-19 years of age in the US from 5% in 1980 to 21% in 2012.[2,3] The 2013 National Basic Health Research (*Riset Kesehatan Dasar/RISKESDAS*) data showed that the prevalence of overweight and obesity in adolescents aged 13-15 years was 10.8%, consisting of 8.3% overweight and

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2.5% obese. *The 2013 RISKESDAS* data showed that the highest prevalence of obesity was in the 13-15-year age group nationally, while the prevalence of obese adolescents in Denpasar, Bali, was 15.2%.[4]

High obesity rates in adolescents also increases the risk of closely related diseases, one of which is cardiovascular disease. A study in 2002 showed that obese adolescents had three times higher risk of experiencing hypertension compared to non-obese adolescents.[5] Obesity can lead to changes in heart structure and function which can increase the risk of cardiovascular disorders in adults. Several studies found a close relationship between obesity and cardiovascular disorders including hypertension, arrhythmia, left atrial enlargement, and decreased systolic and diastolic function.[6,7]

The correlation between obesity and ECG abnormalities has been studied by several researchers. Obesity is considered closely related to an increase in resting heart rate, as well as elongation of the PR interval and duration of QRS.[6,8] Another study showed a correlation between obesity and elongation of the QT interval and leftwards displacement of the heart axis.[6] A study performed in Manado, North Sulawesi, showed an increase in left ventricular hypertrophy in children with obesity, but the result was not statistically significant.[9] A Vienna, Austria study showed that decreasing the weight of obese children and adolescents could change their electrocardiographic findings, decrease heart rate, and shorten QT interval.[10] A study in Lagos, Nigeria showed no statistically significant differences between obese adolescents and controls, but the study revealed that 9 of 49 (18.3%) obese adolescents presented with prolonged QTc.[11] Studies regarding obesity and ECG abnormalities were mostly performed in adults, and only a few was performed in adolescents and children. Data regarding electrocardiographic image in obese adolescents is still lacking, especially in Indonesia.

Electrocardiographic examination is easy, inexpensive, non-invasive, and readily available in all healthcare facilities in every city and district in Indonesia. This examination may be useful as the initial screening tool to detect heart abnormalities in obese adolescents, thus early detection and intervention can be achieved before heart structure changes become irreversible.[6,8]

Methods

This cross-sectional study was conducted at the Children's Cardiology Clinic - Integrated Heart Center of Sanglah General Hospital, Denpasar, Bali. Obese adolescents from several junior high schools in Denpasar underwent ECGs from December 2016 to April 2017.

Inclusion criteria were obese adolescents aged 11 to 15 years, who were willing to participate in the study and provided informed consent. The exclusion criteria were those with a history of chronic diseases, receiving drugs that were known to interfere with cardiac or respiratory function, or a history of chronic alcohol or tobacco consumption. The minimum sample size was determined to be 76 subjects, using a sample calculation formula for categorical descriptive data.

Obesity in adolescents was defined as body mass index (BMI) more than 2 standard deviations (SD) based on the 2007 WHO BMI-for-age curve.[11] Subjects were classified as obese if the BMI was within 2-3 SD, and severe obesity if the BMI was more than 3 SD. QTc interval prolongation is the prolongation of the QT interval after corrected heart rate, which can be evaluated through ECG examination. The interval value of QTc > 0.44 seconds was considered to be prolonged. Prolonged PR interval was the extension of the PR interval. If the value of the PR interval exceeded the limit according to age and heart rate, it was considered to be prolonged.

All subjects underwent a thorough cardiac evaluation including past medical history, physical examination, anthropometric evaluation, and 12-lead ECG examination. The same 12-lead ECG (*Fukuda Denshi*®) was used for all subjects. A paper speed of 25 mm/s and an amplitude of 10 mm/mV were used. The ECGs were recorded and analyzed by the same physicians throughout the study and confirmed by a pediatric cardiologist.

Data obtained were analyzed with *SPSS ver. 20.0* software using descriptive analysis for subjects' characteristics and variables studied. Sample characteristics included in categorical variables were shown as number (n) and percentage (%), while numerical variables were shown as mean (SD). This study was approved by the Research Ethics Committee at Universitas Udayana Medical School, Sanglah Hospital, Denpasar.

Results

We analyzed a total of 78 ECGs of obese adolescents (60% male). Subjects' mean age was 13.2 (SD 1.0) years. Most subjects were classified as obese [58 (68%)], while 25 (32%) subjects were classified as severe obesity. Pre-hypertension was found in 25 (32%) subjects, and hypertension was found in 18 (23%) subjects. Eight (10%) subjects had stage 2 hypertension. Characteristics of subjects are shown in **Table 1**.

Table 1. Subjects' characteristics

Clinical characteristics	(N=78)
Mean age (SD), years	13.2 (1.04)
Gender, n (%)	
Male	47 (60)
Female	31 (40)
Mean weight (SD), kg	82.6 (15.16)
Mean height (SD), cm	161.3(8.3)
Mean BMI (SD), kg/m2	31.5 (4.11)
Mean BMI Z-score (SD)	2.8 (0.6)
Nutritional status, n (%)	
Obese	53 (68)
Severe obesity	25 (32)
Blood pressure, n (%)	
Normotensive	35 (45)
Pre-hypertension	25 (32)
Hypertension grade I	10 (13)
Hypertension grade II	8 (10)

There were 29 (37%) subjects with abnormal ECGs. Sinus tachycardia was present in 13 (17%) subjects, and sinus arrhythmia was identified in 11 (14%) subjects. The mean QTc interval was 0.40 (SD 0.03) seconds, 8 (10%) patients experienced prolongation of the QTc interval. The mean PR interval was 0.156 (SD 0.02) seconds; five (6%) patients presented with prolongation of PR interval. There were no shifts of the P wave, QRS wave and T wave axes, changes of P wave morphology, low QRS voltage, T wave flattening, ventricular enlargement, or ST segment changes found in this study. Complete ECG characteristics are presented in **Table 2**.

Table 2. Electrocardiogram results

ECG characteristics	(N=78)
Rhythm, n (%)	
Normal sinus rhythm	53 (68)
Sinus tachycardia	13 (17)
Sinus bradycardia	1 (1)
Sinus arrhythmia	11 (14)
Mean heart rate (SD), beats/min	89.36 (16.4)
Mean PR interval (SD)	0.156 (0.02)
Prolonged PR interval, n (%)	5 (6)
Mean QRS duration (SD)	0.08 (0.10)
Mean QTc interval (SD)	0.40 (0.03)
Prolonged QTc interval, n (%)	8 (10)
Mean P wave amplitude (SD), mV	1.07 (0.34)
Mean P wave duration (SD), seconds	0.08 (0.01)
Normal ST segment, n (%)	78(100)
T wave, n (%)	
Normal T wave	78 (100)
ECG abnormalities, n (%)	29 (37)

Discussion

Obesity in adolescents is a known risk factor for cardiovascular disease, mortality, and sudden cardiac death. Studies in adults have reported several ECG phenomena associated with obesity, but data in children and adolescents are limited. The prevalence of heart abnormalities detected by ECG among healthy obese adolescents in our study was 37%, which was higher than a previous study (27%).[12] The difference may have been due to the subject populations, as the previous study by Sadoh *et al.* studied both overweight and obese adolescents, while we selected only obese adolescents as subjects.[12]

Abnormal heart rhythm, prolonged PR interval, and prolonged QTc interval were the three most common ECG abnormalities found in our study. Studies in adults have reported that various ECG changes were associated with obesity. These include left axis deviation, signs of left ventricular hypertrophy, bradycardia, and alterations of cardiac repolarization, like ST segment depression or T-wave inversion.[13,14] There are several hypotheses concerning possible influencing factors of obesity on ECG parameters. For example, increased cardiac output, thickening of epicardial and subcutaneous adipose tissue, influence of the autonomic nervous system, or hormonal and electrolyte disturbances may affect ECG parameters.[15]

Normal sinus rhythm refers to normal regular rhythm of the heart which is set by the sinoatrial node and may be assessed by ECG.8 Most subjects

in this study had normal sinus rhythm (68%). Sinus tachycardia occurred in 13 (17%) patients. This finding may have been due to anxiety, increased sympathetic activity, metabolic demand, and/or cardiac output. Other studies comparing obese and lean subjects have reported no significant difference in heart rate, but have reported higher heart rates in obese than in lean individuals. These differences rarely exceeded seven beats per minute and were not clinically important in most cases. Heart rate in obese subjects decreases with weight loss due to decreases in cardiac output, which is elevated in obesity, and occurs primarily because of a decline in stroke volume.[16]

Sinus arrhythmias occurred in 11 (14%) subjects. Of these, two subjects presented with ventricular extrasystoles (premature ventricular contraction). This finding was higher than previously reported by Frank *et al.* (4.8%).[13] Besides age and demographic differences, we suspect that the high prevalence of sinus arrhythmias in our study was due to the presence of physiological arrhythmias, which are commonly found in young people. This physiological phenomenon is called respiratory sinus arrhythmia. Respiratory sinus arrhythmia is heart rate variability in synchrony with respiration, resulting in shortened R-R interval during inspiration and prolongation during expiration.[17] Several longitudinal studies have demonstrated associations between obesity and cardiac dysrhythmias with increased risk of sudden cardiac death linked to increased adiposity.[17-19] The complex mechanisms leading to increased cardiac arrhythmias in obese individuals remain poorly understood. There has been an increased focus on the pathogenic role of adipose depots in abnormal electrical and structural remodeling leading to increased arrhythmogenicity in obese's hearts.[18]

Five (6%) subjects had prolongation of their PR interval. This finding was in agreement with a previous study that showed both overweight and obese children had significantly longer PR intervals compared to a control group of normoweight kids.6 Prolonged PR interval has been associated with an increased risk of heart failure, atrial fibrillation, and mortality.[6] Prolonged PR interval is also correlated with endothelial dysfunction and activation of vascular repair.[20] The PR interval tends to progressively increase with BMI and waist circumference. For each 5 unit increase in BMI, the PR interval increased by 2.4 ms. These results suggest that atrial remodeling (such as atrial fibrosis or autonomic remodeling) may occur in obesity.[21]

We found that 8 (10%) patients experienced prolonged QTc interval. This finding was similar to other studies which concluded that obesity was associated with QTc interval prolongation.[13,22,23] A study showed that the QTc interval of obese patients was longer than that of controls.[24] In contrast, other studies suggested that an association of obesity and QTc interval remained controversial.[6] The majority of previous studies had adult subjects, while the possible correlation between obesity and prolonged QTc interval in adolescents has not been studied in detail on a large-scale. The QT interval on the ECG represents depolarization and repolarization of the ventricles and is measured from the onset of the QRS complex to the end deflection of the T wave.[24] The QT interval is modulated by autonomic function, therefore, it is often corrected to be independent of heart rate. In our study, we used Bazzett's formula ($QTc = QT/\sqrt{RR}$) to correct the QT interval.[25] Prolonged QTc interval has been associated with life-threatening arrhythmias and an increased risk of sudden cardiac death.[23,25] Therefore, it is important to assess QTc intervals, especially in high-risk subjects.

There was no ventricular enlargement found in our subjects. This finding was consistent with previous studies.[6,9] In contrast, the evidence in adults presented so far supports the findings that the likelihood of LVH is higher in obese individuals. As such, obesity may increase the risk of LVH development over time.[9] The reason for the discrepancies among these studies is uncertain. The ECG criteria for left and right ventricular hypertrophy have a poor sensitivity, but strong specificity, in obese subjects. Thus, ECG has very limited use in the diagnosis of ventricular hypertrophy in obese subjects. Another theory suggested that the presence of ventricular hypertrophy in obese adults was associated with prolonged exposure to obesity, hypertension, diabetes mellitus, and dyslipidemia.[26]

Obesity in childhood and adolescence is considered to be a risk factor for death from cardiovascular disease and from all causes in adulthood. The Prospective Studies Collaboration analyzed data from 57 prospective studies with almost 900,000 participants. This study result showed that each 5 kg/m^2 BMI increment was associated with

about 40% higher vascular mortality (HR=1.41; 95% CI 1.37 to 1.45).[27]

A limitation of our study was its cross-sectional design, such that the associations between obesity and ECG parameters could be described, however, the long-term interaction between obesity and ECG variables was not analyzed. In addition, we did not evaluate serum electrolytes and markers of metabolic disturbance (for example, lipid profile and serum glucose) that could influence the ECG results. Subject were also not evaluated by echocardiography, which would have assessed cardiac function. Furthermore, the ECG changes in this study were relatively small, and it was unclear whether these ECG abnormalities had clinical significance. These issues require more investigation, as well as further and larger follow-up studies.

Electrocardiographic examination is a cost-effective method for detecting cardiac abnormalities. Periodic ECG examination of obese adolescents is recommended for early diagnosis and intervention to decrease the chances of sudden cardiac death and cardiovascular events. In conclusion, the prevalence of cardiac abnormalities based on ECG examination in obese adolescents was 37.2%, consisting of abnormal heart rhythm, prolonged PR interval, and prolonged QTc interval.

Conflict of Interest

None declared.

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Original Article

Cystatin C level and amikacin use in neonatal sepsis

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Abstract

Background Amikacin is the antibiotic of choice for eradicating bacteria in neonatal sepsis because of its effectiveness against Gram-negative bacteria. However, this drug has nephrotoxic effects. Monitoring kidney function in neonates is very important because amikacin can interfere with development of the kidney. Several studies have shown that serum cystatin C levels were closer to glomerular filtration rate (GFR) values compared to serum creatinine levels.

Objective To evaluate cystatin C levels before and after administration of amikacin in neonates with sepsis.

Methods This prospective cohort study was conducted in one group with a pretest and posttest design. Thirty neonatal sepsis patients who received amikacin therapy at Sanglah General Hospital, Denpasar, Bali, were included by consecutive sampling. Their cystatin C levels were measured before and after receiving amikacin therapy. Data were normally distributed and analyzed by paired T-test, with a value of $P<0.05$ considered to be significant.

Results The mean difference was 0.23 [1.57 (SD 0.29) *vs.* 1.80 (SD 0.28)] mg/L with P value < 0.001. There was different value of cystatin c level before and after amikacin therapy with deviation standard 0.25 with $P<0.001$ (alfa 5%).

Conclusion Cystatin C levels are significantly higher in neonates with sepsis after administration of amikacin. **[Paediatr Indones. 2020;60:1-5; doi: http://dx.doi.org/10.14238/pi60.1.2020.1-5]**.

Keywords: *neonatal sepsis; cystatin C; renal function*

Neonatal sepsis remains a worldwide health problem. Mortality and morbidity due to neonatal sepsis are high. Neonatal sepsis can lead to complications depending on its severity or the level of impairment of the organs involved. Data from the *World Health Organization* (WHO) estimated that neonatal sepsis causes four million deaths every year. The neonatal mortality rate (death in the first 28 days of life) is 34 per 1,000 live births, and 98% of these are from developing countries, including Indonesia.[1] The high rate of morbidity and mortality due to bacterial infection suggests that antibiotics be given immediately after an infection is suspected.[1,2]

Neonates with suspected sepsis, antimicrobial therapy must be given immediately.[3-5] Amikacin is drug of choice for neonatal sepsis but has a narrow therapeutic index which can be toxic to the kidney.[6-8] Monitoring kidney function in neonates is very important where at that time is an important period

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of kidney development.[9-10] Several studies have shown that serum cystatin c levels are closer to GFR values compared to serum creatinine levels,[11-22] but information on serum cystatin c in populations based on age especially neonates is still rare. We aimed to evaluate cystatin C levels before and after administration of amikacin in neonates with sepsis.

Methods

This was a prospective cohort, single group study with a pretest and posttest design. It was conducted in the Neonatal Ward (levels 2 and 3) of Sanglah Hospital, Denpasar, Bali, from August 2017 to August 2018. Examination of cystatin C levels was carried out in Prodia Laboratory, Denpasar, on the 10th days of amikasin dose. Subjects were septic neonates who received amikacin 7.5 mg/kg/dose for 10 days.[23-28] They were included by consecutive sampling until the minimum required sample size was met. Inclusion criteria were neonates with a gestational age of ≥ 35 weeks, early-onset neonatal sepsis (EONS) or late-onset neonatal sepsis (LONS). Neonates with major congenital abnormalities, history of severe asphyxia at birth, as well as those who received amikacin or other nephrotoxic drugs (furosemide, vancomycin) before starting the study, those who did not complete the course of antibiotics due to discharge against medical advice, or those who died were excluded from this study. Calculation of the minimum required sample size was done with a sample formula to test the hypotheses against two mean groups in pairs, with $\alpha=0.05$, $\beta=0.10$, standard deviation of 0.5, and minimum average difference of 0.3, resulting in 29 subjects. To account for a 10% probability of loss to follow-up, the final sample size was deemed to require a minimum of 32 neonates.

The independent variable was amikacin therapy. The dependent variable was serum cystatin C level. The random variable was the adequacy of fluid intake. Data were analyzed using *SPSS software*. Subjects' characteristics are presented descriptively in the form of tables and narratives. Data normality was analyzed using Shapiro-Wilks test ($P>0.05$). Comparison of mean cystatin C levels before and after amikacin therapy was done with paired T-test. The analysis results are displayed in table form.[29]

Results

During the study period, 30 patients was included in data analysis (2 samples was loss to follow up), half of them were male. The flow of subject recruitment is shown in **Figure 1**. Subjects' mean chronological age was 2 (SD 1.5) days, mean birth weight was 2,995 (SD 250.3) grams, and mean weight at admission was 2,991 (SD 248.5) grams. Of the 30 subjects, 21 were delivered by Caesarean section, 25 had EONS, and 27 received parenteral nutrition. In addition, 17/30 subjects used mechanical ventilators, 10/30 subjects used CPAP, and 3/30 subjects used high flow oxygen. Subjects' mean urine production during observation was 2.0 (SD 0.6) mL/kg/hour, with an average intake of 126.67 (SD 12.1) mL/kg/day.

The Shapiro-Wilk normality test had a value of $P=0.8$ with alpha 5%, hence the data were normally distributed. Clinical and septic work up performed every 3 days; during the treatment period, 18 patients

Table 1. Subjects' characteristics

Characteristics	(N=30)
Gender, n	
Male	15
Female	15
Age, n	
1-3 days	25
4-7 days	5
Median age (range), days	2 (1-7)
Mean birth weight (SD), grams	2,995 (250.3)
Mean weight at admission (SD), grams	2,991.67 (248.5)
Method of delivery, n	
Vaginal	9
Caesarean section	21
Type of sepsis, n	
Early-onset	25
Late-onset	5
Type of nutrition, n	
Parenteral	27
Enteral	3
Breathing assisted device, n	
Mechanical ventilator	17
CPAP	10
High flow oxygen	3
Mean urine output (SD), mL/kg/hour	2.01 (0.63)
Mean fluid intake (SD), mL/kg/day	126.67 (12.13)
Mean hemoglobin (SD), g/dL	16 (1.34)

showed clinical and laboratory improvement, those amikacin course was stopped on the day 7. For the rest 12 patients, amikacin course was stopped on day 10. Paired T-test revealed that mean cystatin C levels were significantly higher after amikacin treatment than before treatment. The difference in mean value before and after the administration of aminoglycosides was 0.23 (SD 0.25) mg/L, (P <0.001) (**Table 2**). Cystatin C levels after administration of amikacin were significantly different, either evaluated on day 7 or day 10 (**Table 3**).

Discussion

We included only full-term neonates because the formation of nephrons is complete by 35 weeks of gestation, and new nephrons are not formed after birth. Disturbances in the urinary system, such as infection, reflux, or exposure to nephrotoxic substances after this period can interfere with kidney growth.[30-33] The difference in mean values before and after amikacin therapy was 0.232 (SD 0.25) mg/L; (P<0.001); at 5% alpha. Mean cystatin C level was significantly higher

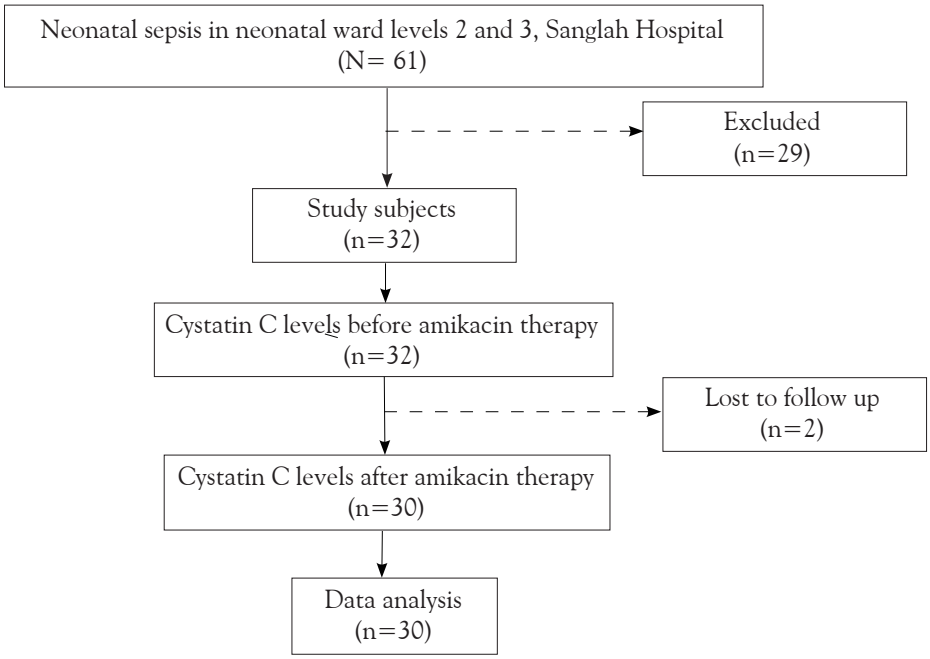

Figure 1. Flow chart of the study design

Table 2. Mean difference in cystatin C levels before and after amikacin therapy

Cystatin C	N	Mean (SD), mg/L	Mean difference	95%CI	P value
Pre-therapy	30	1.57 (0.29)	0.23	0.1439 to 0.326	<0.001
Post-therapy	30	1.80 (0.28)			

Table 3. Cystatin C levels on day 7 and day 10 post-amikacin therapy

Cystatin C		N	Mean (SD)	Mean difference	95%CI	P value
Day 7	Pre-therapy	18	1.55 (0.25)	0.23	0.078 to 0.311	0.017
	Post-therapy	18	1.75 (0.23)			
Day 10	Pre-therapy	12	1.59 (0.34)	0.28	0.111 to 0.464	0.019
	Post-therapy	12	1.87 (0.32)			

after administration of amikacin. Cystatin C levels at the beginning of life are about 1.17 mg/L, normally decreasing at 3-5 days of age. The standard level at 1 year of age is 0.51-0.95 mg/L.[34-38] In healthy neonates, the highest level of cystatin C was detected after birth, and was higher than maternal cystatin C levels [mean maternal cystatin C level (SD) 1.00 (0.20)].[12,38] This finding suggests that cystatin C does not pass through the placenta.[39] Mean cystatin C levels in our study increased on day 7 or 10 after receiving amikacin therapy. This showed as an early marker that there was an abnormality in neonatal kidney function. In neonates with acute renal failure, a difference of 0.3 mg/L in mean cystatin C levels was observed on the first and third day of amikacin therapy.[38] Another study examined creatinine levels of neonatal subjects with sepsis who received aminoglycoside therapy, especially amikacin, before and after therapy (therapy given for 7-10 days) and found no increase in creatinine levels after therapy.[11,38] This study did not compare cystatin c levels with other modalities for renal function examination.

In conclusion, there is a significant difference in cystatin C level before and after administration of amikacin in patients with neonatal sepsis.

Conflict of Interest

None declared.

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Original Article

Blood count to determine chronic inflammation severity in obese adolescents

Martini Wongkar, Handoko Lowis, Sarah M. Warouw, Julius Lolombulan, Stefanus Gunawan

Abstract

Background Obesity is a growing public health problem of rapidly increasing prevalence in developing countries. Chronic low-grade inflammation plays a key role in the pathophysiology of obesity. Blood count values and ratios have been used as markers of inflammatory diseases. These parameters may be useful to determine the severity of chronic inflammation in obese children.

Objective To determine if red blood cell distribution width (RDW), neutrophil-to-lymphocyte ratio (NLR), mean platelet volume (MPV), platelet distribution width (PDW), and platelet-to-lymphocyte ratio (PLR) can be useful for determining the severity of chronic inflammation in obese children.

Methods This study was conducted in obese adolescents aged 14-18 years at senior high schools in Manado, North Sulawesi, from July to September 2018. Students with congenital anomalies, autoimmune diseases, history of asthma, or malignancy were excluded. Pearson's correlation was used to analyze for potential relationships between obesity and red blood cell distribution width (RDW), neutrophil-to-lymphocyte ratio (NLR), mean platelet volume (MPV), platelet distribution width (PDW), and platelet-to-lymphocyte ratio (PLR).

Results There was a negative relationship between obesity and MPV, but it was not statistically significant (r=-0.006 ; P=0.485). There were positive, but not significant relationships between obesity and RDW (r=0.139; P=0.192), NLR (r=0.155; P=0.166), PDW (r=0.02; P=0.45), and PLR (r=0.146; P=0.181).

Conclusion The RDW, NLR, MPV, PDW, and PLR values are not significantly associated with severity of obesity in adolescents. **[Paediatr Indones. 2020;60:6-12; doi: http://dx.doi.org/10.14238/pi60.1.2020.6-12]**.

Keywords: *obese; blood count; adolescents*

Obesity in children has reached epidemic levels in both developing and developed countries, and is known to have an impact on physical and psychological health. One-third of obese children become obese adults and are more likely to experience chronic inflammatory diseases, such as diabetes mellitus and cardiovascular disease at a young age. In Indonesia, the prevalence of childhood obesity is 11.5%, with overweight and obesity in urban areas twice those in rural areas (10.7 and 5.1%, respectively).[1]

A biomarker is a variable used as an indicator of biological or nutritional conditions. Increased levels of high-sensitivity C-reactive protein (hsCRP) were detected in obese people and were a useful marker of cardiovascular risk for obese children. Because of racial and ethnic variations, a limit of 3 mg/L, based on *Center for Disease Control* (CDC)/*American Heart Association* (AHA), cannot be applied to all people, thus other tests are needed to show inflammatory

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status.[2] Laboratory examinations from routine blood specimens are fast, easy, and inexpensive. The red blood cell distribution width (RDW), neutrophil-to-lymphocyte ratio (NLR), mean platelet volume (MPV), platelet distribution width (PDW), and platelet-to-lymphocyte ratio (PLR) are known as inflammatory markers of complete blood counts.[3]At present, there is no laboratory examination to reliably assess chronic inflammation severity. Red blood cell distribution width is a novel biomarker that describes multiple physiological disorders associated with atherosclerosis and coronary heart disease. The ratio between the number of neutrophils and lymphocytes (neutrophils lymphocytes ratio/NLR) is a simple, cost-effective, and profitable inflammation marker which has been studied in many inflammatory diseases, cardiovascular diseases, and cancer. Mean platelet volume, a component of the routine complete blood count, is a marker of platelet function and activity. Decreased MPV levels have been reported as good indicators of disease activity and inflammatory burden in various inflammatory diseases. Platelet distribution width shows differences in platelet size in circulation, while PLR is the ratio between the number of platelets and lymphocytes. These are novel inflammatory markers, which has been demonstrated to be the predictors of various cardiovascular diseases and tumors.

We aimed to determine if RDW, NLR, MPV, PDW, and PLR can be useful for determining the severity of chronic inflammation in obese adolescents.

Methods

This was an observational study in the form of a correlation with a cross-sectional approach. The study was conducted in senior high schools in Manado from July to September 2018. Laboratory tests were conducted in a Prodia laboratory, a private laboratory. The study subjects were comprised of obese adolescents aged 14-18 years who met the following inclusion criteria: diagnosed with obesity, healthy, and whose parents were willing to sign an informed consent. Exclusion criteria were subjects with congenital abnormalities, autoimmune diseases, history of asthma, or malignancy. The sample size was 41 children, selected by two-stage random sampling. The first stage was a simple randomization from all senior high schools to find the number of high schools representing the population. The sampling stage was carried out by simple random sampling from selected high schools.

The independent variable was obesity, and the dependent variables were RDW, NLR, MPV, PDW, and PLR. Obesity was defined as an abnormality or disease characterized by excessive accumulation of body fat. In our study, body mass index (BMI) was calculated based on commonly used guidelines, according to the *Centers for Disease Control* (CDC) 2000 according to age, sex, weight, and height.[4] Obesity was classified as BMI above the 95% percentile. Obesity severity was determined by a comparison of measured BMI and BMI in the 95th percentile, expressed as a percentage.

Subjects underwent complete blood count examinations, which included the five pertinent parameters. The RDW was an indicator of variation in size and volume of red blood cells,[5] NLR was the ratio of absolute neutrophil count (ANC) to absolute lymphocyte count (ALC),[6] PDW showed differences in platelet size in the circulation,[7] MPV determined platelet function and was a new risk factor in determining atherothrombosis,[8] and PLR was the ratio of platelet count over ALC.[9]

This study was approved by the Health Research Ethics Committee from RSUP Prof. Dr. R.D. Kandou and the Manado City Education Office. Descriptive analyses were shown in the form of tables and graphs. Analysis of possible correlations between obesity and RDW, NLR, MPV, PDW, and PLR was by Pearson's correlation. Results with P values <0.05 were considered to be significant. Data processing was done with *SPSS 25* software.

Results

During the study period, 41 obese children fulfilled the inclusion criteria, consisting of 25 (61%) males and 16 (39%) females. Subjects' mean age was 16.2 years, with a range of 14.33 to 18.33 years. Subjects' mean weight was 80.28 kg, ranging from 64.5 to 109 kg, and mean height was 159.92 cm, ranging from 148 to 180 cm. Mean BMI percentile was 111.68% of the 95th percentile ranging from 100 to 141.2% (**Table 1**).

Table 2 shows the mean, median, minimum, and maximum of the complete blood counts and ratios from the laboratory examinations.

Potential relationships between obesity and RDW, NLR, MPV, PDW, and PLR were analyzed by Pearson's correlation coefficient. A negative relationship trend was observed between obesity and MPV, but it was not significant (r=-0.006, P=0.485) (**Figure 1**).

Pearson's correlation analysis revealed positive but not significant correlations between obesity and RDW, NLR, PDW and PLR (r=0.139, P=0.192; r=0.155, P=0.166; r=0.02, P=0.45; and r=0.146, P=0.181, respectively). The scatter diagrams for each laboratory parameter are shown in **Figures 2, 3, 4,** and **5**.

Table 1. Basic anthropometric characteristics of subjects

Characteristics	Mean	Median (range)
Age, years	16.2	16.25 (14.33-18.33)
Weight, kg	80.28	77 (64.5-109)
Height, cm	159.92	160 (148-180)
BMI/age* %	111.68	107.8 (100-141.2)

*comparison of measured BMI and BMI in the 95th percentile according to age

Table 2. Laboratory results

Parameters	Mean	Median (range)
Hemoglobin, gr/dL	12.1	15.1 (12.7-17.9)
Hematocryte, %	44.4	45.3 (36.7-51.5)
Erythrocytes, 10^6/mm^3	5.4	5.4 (4.3-6.4)
Leukocytes, cells/mm^3	10,398	10,400 (5,500-18,200)
Thrombocytes, /mm^3	361,926	356,000 (230,000-506,000)
RDW, %	12.7	12.6 (11.5-14.9)
NLR	1.7	1.7 (0.8-2.8)
MPV, fL	9.8	8.8 (8.4-11.4)
PDW, fL	10.9	10.6 (8.7-14)
PLR	113.8	107 (55-215)

Discussion

Obesity is defined as a disorder characterized by excessive accumulation of body fat. Obesity has various sequelae related to health, including chronic inflammation.[10] Several blood count values and ratios have been used as indicators of chronic inflammation.[11] A previous study compared RDW in 139 morbidly obese children, 28 overweight children and 82 healthy children of normal weight. This study showed that RDW was significantly higher in

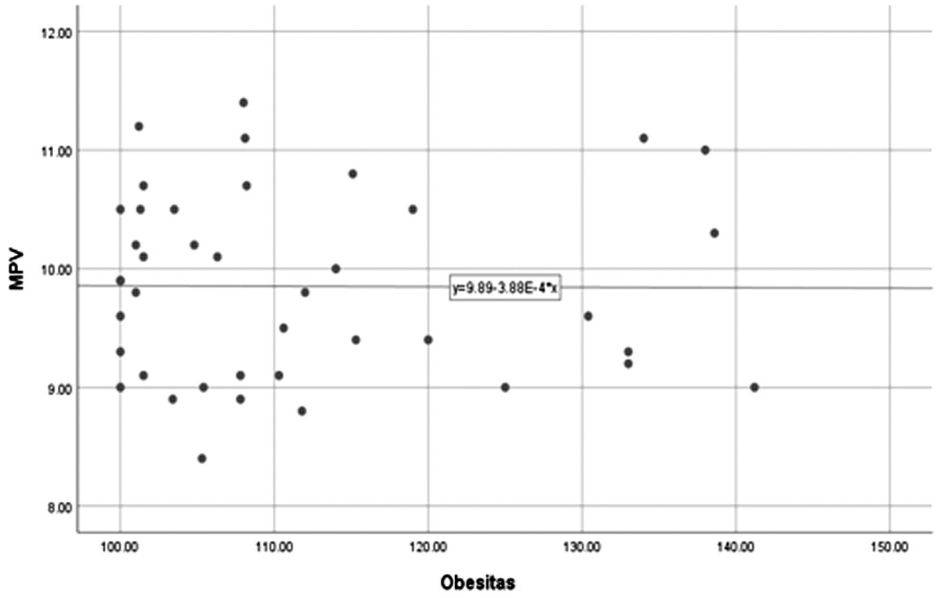

Figure 1. Scatter diagram of the analysis of severity of obesity and MPV

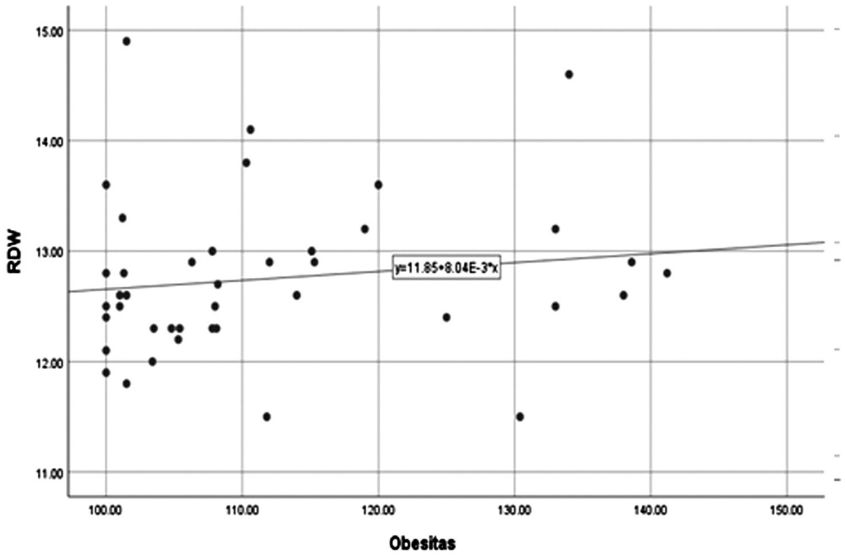

Figure 2. Scatter diagram of the analysis of severity of obesity and RDW

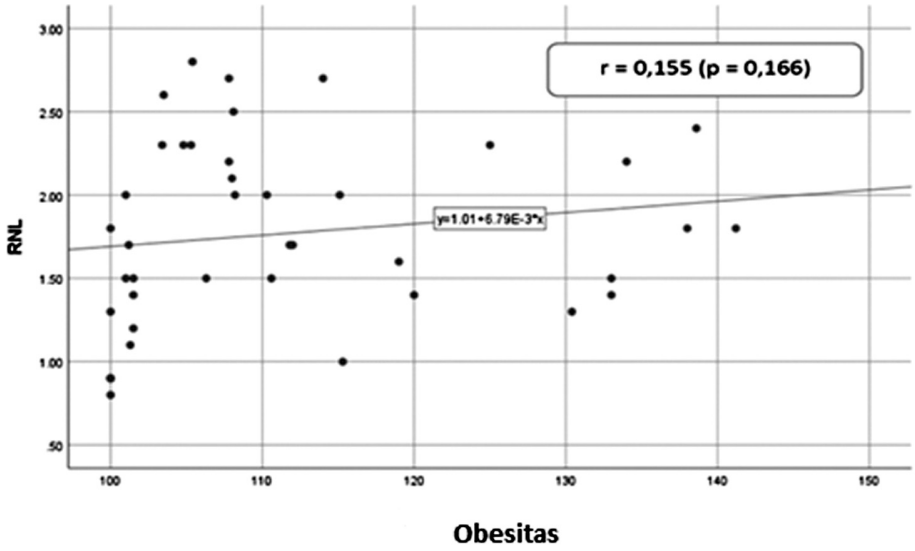

Figure 3. Scatter diagram of the analysis of severity of obesity and NLR

overweight and obese children compared to those with normal weight.[12] The RDW is a novel biomarker that describes multiple physiological disorders associated with atherosclerosis and coronary heart disease.[13] Our study also found the same trend between RDW value and the severity of obesity.

The NLR was also found to be a potent marker of inflammation in children.[14,15] PA previous study assessed the inflammatory status of obese children using NLR. Of 130 obese children (aged 6-15 years) and 57 control children with normal weight (aged 7-15 years), a significant increase in neutrophils, lymphocytes, CRP, and NLR was found in the obese group compared to the control group.[16] Findings in our study show the same possible positive relationship in our study where more increased in NLR found in more severe obesity.

Platelets' role in systemic inflammation have

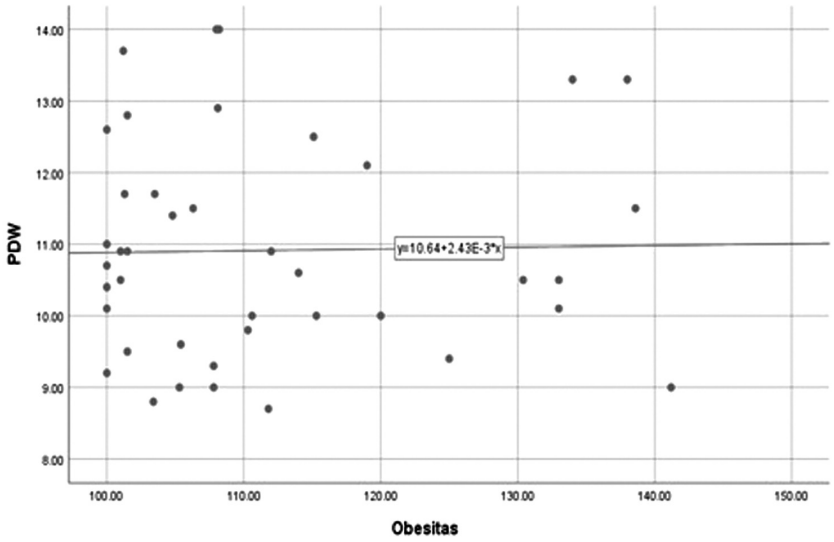

Figure 4. Scatter diagram of the analysis of severity of obesity and PDW

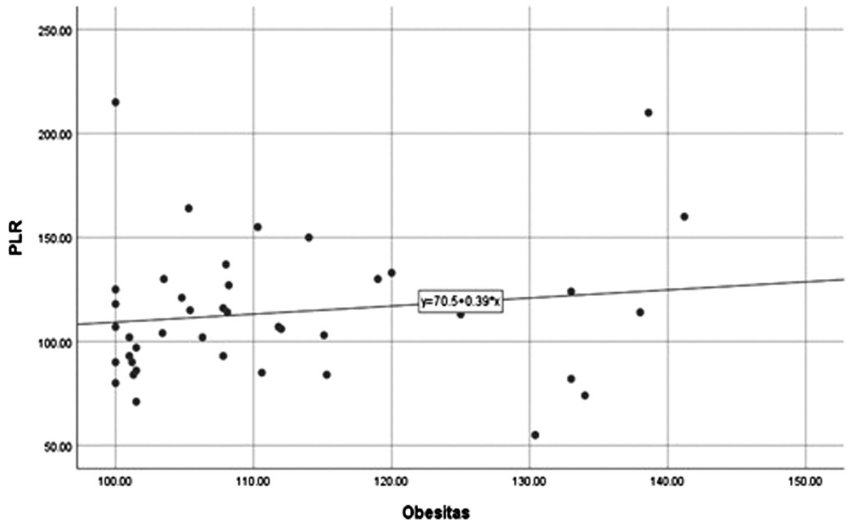

Figure 5. Scatter diagram of the analysis of severity of obesity and PLR

been reported in several studies. Level of MPV has been found associated with low grade inflammation.[17] A high PDW levels found to be caused by swelling, destruction, immaturity, and high reactivity of platelets.[18] Another study reported that MPV and PDW concentrations were significantly higher in the obese group than in the normal weight control group.[19] In obese individuals, although the mechanism underlying the increase was unclear, procoagulant status may have been induced by adipocytokines such as leptin, adiponectin, resistin, and PAI.[19] Discrepancies in MPV value still being found in recent studies.[18] Our study did not show increased MPV value parallel with severity of obesity, although PDW show a positive trend.

Another study found a positive correlation between PLR and the homeostasis model assessment of insulin resistance (HOMA-IR), potentially derived from a proinflammatory status associated with obesity from the complex interactions between platelets, insulin signaling and inflammation.[20] Chronic inflammation is a strong risk factor for many obesity-

related diseases. Increased inflammation, shown in a decrease in adiponectin and an increase in proinflammatory cytokines, found in a longer period of obesity.[21]

The limitation of this study was not including confounding factors such as age of onset and duration of obesity, family history of obesity, diabetes mellitus, and cardiovascular disease that may influence severity of obesity and the various components of complete blood counts.

In conclusion, RDW, NLR, MPV, PDW, and PLR values are not significantly associated with severity of obesity in adolescents.

Conflict of Interest

None declared.

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Original Article

Umbilical arterial profiles as predictors of severity of hypoxic ischemic encephalopathy after perinatal asphyxia

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Abstract

Background Perinatal hypoxic-ischemic encephalopathy (HIE) remains a major cause of neurodevelopmental impairment. Umbilical cord blood analysis provides an objective assessment of newborn metabolic status. Accordingly, it is recommended that physicians attempt to obtain venous and arterial samples when there is high risk of neonatal compromise.

Objective To compare the predictive value of umbilical arterial blood pH, lactate and base deficit for subsequent development of severity of hypoxic ischemic encephalopathy (HIE) after perinatal asphyxia and comparison of these parameters to determine which one is superior in predicting severity.

Methods Umbilical cord arterial blood of newborns with perinatal asphyxia was tested for pH, lactate, and base deficit estimation. These newborns were evaluated in level III NICU and divided into two groups. Group 1 had no or signs and symptoms of HIE I and group 2 had signs and symptoms of HIE II/III. Values of pH, lactate, and base deficit were tabulated and analyzed by receiver-operating characteristic curves. Optimal cut-off values were estimated based on the maximal Youden index.

Results Mean pH was significantly lower in group 2 than in group 1, while lactate and base deficit were significantly higher in group 2 than in group 1. Cut-off points for determining severity of HIE were pH <7.13, lactate >6.89 mg/dL, and base deficit >7 mEq/L. Sensitivity and specificity for these cut-off points were 100% and 91.49% for pH, 100% and 85.11% for lactate, and 82.4% and 91.76% for base deficit, respectively. Predictive abilities of all three parameters were similar in determination of HIE severity.

Conclusion Umbilical arterial pH, lactate, and base deficit have excellent accuracy to predict the severity of HIE. All three parameters have similarly good predictive ability. **[Paediatr Indones. 2020;60:24-30; doi: http://dx.doi.org/10.14238/pi60.1.2020.24-30]**.

Keywords: *perinatal asphyxia; pH; lactate; base deficit; HIE; umbilical arterial*

Perinatal asphyxia is an insult to the fetus or newborn due to lack of oxygen and/or perfusion to the brain and other organs. It is often associated with multiple pathophysiologic consequences which lead to multiorgan dysfunction.[1] Perinatal asphyxia can lead to myocardial dysfunction, rhythm abnormalities, acute renal failure, metabolic abnormalities (hypoglycaemia, hyperglycaemia, hypocalcaemia),[2] respiratory failure, necrotising enterocolitis in preterm infants, and coagulation abnormalities. Moreover, hypoxia and decreased perfusion lead to devasting immediate and long term complications of the central nervous system (CNS). Hypoxic-ischemic encephalopathy (HIE) is one such complication, and is among the leading causes of neonatal brain injury, morbidity, and mortality.[3] Severe HIE may have a deleterious impact on newborns, subsequently leading to cerebral palsy, refractory seizures, or strokes.[4] Intrapartum hypoxic events caused an estimated 717,000 deaths in 2010 (1 in 5 of

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all neonatal deaths worldwide).[4] Newborns surviving HIE have a high risk of developing neuropsychological impairment like psychosis, depressive illness, and cognitive impairment.[5]

The relationships between acidosis, base deficit, and lactate to perinatal asphyxia remain inconclusive and data are limited. Some studies have demonstrated a correlation between degree of acidosis, increased lactate, and increased base deficit with the neonatal neurological outcome.[5-7] Other studies suggested that most neurological outcomes are related to disease rather than perinatal asphyxia itself, especially metabolic status like acidosis, increased lactate level, and base deficit.[5,7,8] However, lactate's correlation with perinatal asphyxia has been extensively studied, but the smaller number of studies on acidosis and base deficit relationships to perinatal asphyxia and potential metabolic predictors for HIE severity has left a gap in understanding about birth asphyxia and its impact on neurological outcome.[8]

Depending on severity, neonates with perinatal asphyxia can completely recover, develop permanent disability, or even expire. Thus, caregivers need to know the prognosis in order to make treatment decisions such as neuroprotective strategies like therapeutic hypothermia, or withdrawal of therapy. As such, we aimed to find accurate predictors of HIE severity after perinatal asphyxia.

Methods

This prospective, observational study was carried out in the level III nursery of the *World College of Medical Sciences* (WCMS) Haryana, India, from June 2017 to November 2018. Subjects' parents provided informed consent. The Ethics and Scientific Committee of our hospital approved the study. Intrapartum monitoring of all newborns was done by Doppler ultrasonography and biophysical profile scoring. Sixty-four full term newborns of >37 weeks gestational age in whom asphyxia was suspected were included in the differential diagnosis when there was:[1]

a. Prolonged (>1 hour) antenatal acidosis
b. Fetal HR <60 beats per minute
c. Apgar score ≤3 at ≥10 minutes
d. Need for positive pressure ventilation for >1 minute or first cry delayed >5 minutes
e. Seizures within 12 to 24 hours of birth
f. Burst suppression or suppressed background pattern on EEG or amplitude-integrated electroencephalogram (aEEG).

Neonates with life-threatening congenital malformation, congenital heart diseases, septicaemia, or intracranial bleeding were excluded from this study. Subjects' umbilical arterial specimens were collected and sent to the lab for measurement of pH, lactate, and base deficit levels. Newborns with perinatal asphyxia were evaluated in the level III NICU by department senior residents who were well trained in neonatal neurological examination. Group 1 infants developed either no or signs or symptoms of HIE stage I, and group 2 consisted of neonates who developed symptoms and signs of HIE stage II and III. HIE staging was done by Sarnat classification,[9] as shown in **Table 1**.

Table 1. Sarnat classification of HIE[9]

Variables	Stage I (Mild)	Stage II (Moderate)	Stage III (Severe)
Consciousness	Hyperalert	Lethargic or obtunded	Stupor or coma
Activity	Normal	Decreased	Absent
Neuromuscular control			
a. Muscle tone	Normal	Mild hypotonia	Flaccid
b. Posture	Mild distal flexion	Strong distal Flexion	Intermittent decerebration
c. Stretch reflexes	Overactive	Overactive	Decreased or absent
Primitive reflexes			
a. Sucking	Weak	Weak or absent	Absent
b. Moro	Strong	Weak incomplete/strong	Absent
c. Tonic neck	Slight		Absent
Autonomic function			
a. Pupils	Dilated	Constricted	Variable, unequal
b. Heart rate	Tachycardia	Bradycardia	Variable

Post-natal management of all newborns with asphyxia was done as per protocol. Ventilation was done with CO2 maintained in the normal range; for oxygenation, O2 levels were maintained in the normal range by supplemental O2 and/or mechanical ventilation. For temperature, passive cooling should be done by turning off warming lights to initiate hypothermia as soon as possible after the HI insult. Unfortunately, therapeutic hypothermia could not be given to our subjects because facility is unavailable in our neonatal intensive care setup. Hyperthermia was strictly avoided. Perfusion, cardiovascular stability, and adequate mean arterial blood pressure was maintained to provide adequate cerebral perfusion pressure. Other physiological metabolic states like electrolyte levels, glucose level, and control of seizures, were maintained by appropriate treatment.

All data were collected and analyzed by *SPSS 22* (*SPSS Inc*., Chicago, Illinois, USA) and *MedCalc 18.11* software. The quantitative variables between the two groups were compared using student's T-test (for independent data) and two-tailed Mann-Whitney U test. Results with P values <0.05 were considered to be statistically significant.

The sensitivity, specificity, positive and negative predictive values, and likelihood ratios were computed by receiver-operating characteristic (ROC) curves. The clinical values (cut-off points) were chosen based on the maximal Youden index, corresponding to the combination of highest sensitivity and specificity determined at the apex of the ROC curves.

Results

Our study included 64 neonates who fulfilled the criteria for perinatal asphyxia. Out of these 64 neonates, 15 developed no symptoms of HIE, 32 developed HIE I, 7 developed HIE II, and 8 evolved into HIE III. Two infants who had HIE III clinical features died. Thus, group 1 consisted of 47 (73.44%) neonates and group 2 had 17 (26.56%) neonates. Mean gestational age, gender, mode of delivery, and mean APGAR scores at 5 and 10 minutes were compared between the two groups, but no significant differences were revealed (**Table 2**).

The pH in both groups was acidic, but mean pH in group 2 (6.94) was significantly more acidic than group 1 (7.25). Mean lactate in group 1 (5.74 mg/dL) was significantly lower compared to the results in group 2 (9.94 mg/dL). Comparison of lactate levels of two groups showed statistically significant difference ($P=0.002$). Mean base deficit was 5.4681mEqs/L and 9.82mEqs/L in group 1 & group 2, respectively. Statistical difference of lactate levels among these groups was also significant ($P=0.001$). Lactate and base deficit levels were significantly higher and mean pH was significantly lower in Group 2 compared to those in Group 1, as shown in **Table 2**.

Using the ROC curve analysis of pH, we derived a cut off value of $pH<7.13$, with 100% sensitivity and 91.49% specificity for predicting perinatal asphyxia to evolve into HIE stage II/III. The ROC curve analysis of base deficit revealed a cut-off value

Table 2. Clinical characteristics of patient groups

Parameter	Group 1 (n=47)	Group 2 (n=17)	P value
Total newborns, n	47	17	
Mean gestational age, weeks	38.2	38.5	0.98
Gender, n Male Female	 25 22	 9 8	 0.786
Mode of delivery Vaginal LSCS	 29 18	 10 7	 0.089
Mean APGAR score at 5 min & 10min (SD)	5 (2)	4 (1)	0.32
Mean pH (SD)	7.25 (0.09)	6.94 (0.17)	0.0001
Mean lactate level (SD), mg/dL	5.74 (1.66)	9.94 (1.43)	0.002
Mean base deficit (SD), mEq/L	5.4681(1.5)	9.82 (2.12)	0.001

>7, with 82.4% sensitivity and specificity 91.76%, for predicting the development of HIE stage II/III in asphyxiated newborns. The ROC curve analysis of lactate revealed a cut-off point of > 6.89 mmol/L to predict development of HIE stage II/III with 100% sensitivity and 85.11% specificity. The ROC curves are shown in Figure 1 and the analysis results for the 3 variables are shown in **Table 3**.

A comparison of ROC curves for all three parameters revealed no significant differences in predictive ability, as shown in **Table 4** and **Figure 1**.

Discussion

Neuropathological (selective neuronal necrosis, parasagittal cerebral injury, periventricular leukomalacia, focal (and multifocal) ischemic brain necrosis, stroke) and neurological clinical syndromes (cerebral palsy, various seizure disorders,) which are essential components of neonatal neurology are usually the sequelae of HIE. Thus, it is important to be vigilant on biochemical and physiological changes due to hypoxia which are predictive of the structural and functional manifestations of encephalopathy,

Table 3. ROC curve characteristics of variables of umbilical artery blood

Test result variable(s): Umbilical artery	Associated criterion (cut-off value)	Area	Std. error	Asymptomatic sign	95%CI		Sensitivity, %	Specificity, %	Positive LR	Negative LR	PPV, %	NPV, %
					Upper bound	Lower bound						
pH	7.13	0.968	.0202	<0.001	0.891	0.996	100	91.49	11.75	0.00	81	100
Base deficit	>7.00	0.951	0.026	<0.0001	0.866	0.989	82.4	91.76	11.98	0.25	77.8	93.5
Lactate	>6.89	0.952	0.0246	<0.0001	0.867	0.990	100	85.11	6.71	0.00	70.8	100

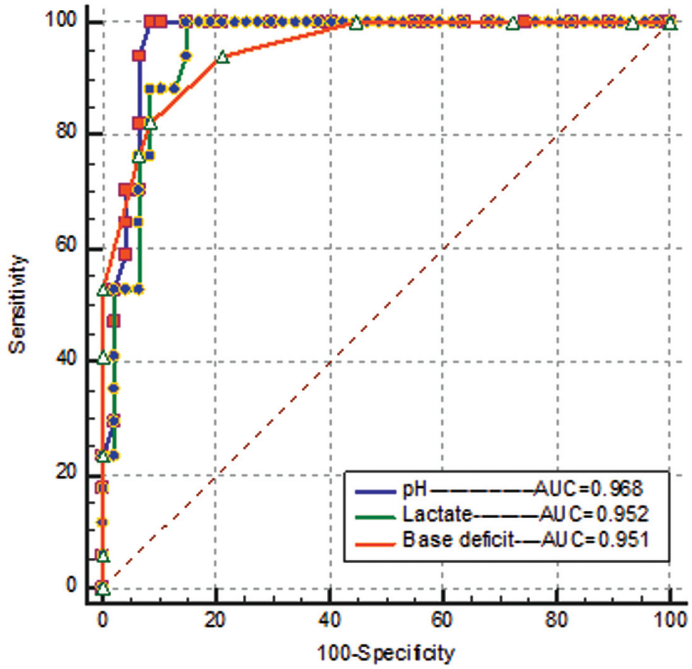

Figure 1. Comparison of ROC curves

Table 4. Pairwise comparison of ROC curves of umbilical arterial blood parameters[10]

Parameters	pH ~ lactate	pH ~ base deficit	Lactate ~ base deficit
Difference between areas	0.0163	0.0169	0.000626
Standard error[10]	0.0289	0.0301	0.0312
95% CI	-0.0404 to 0.0730	-0.0422 to 0.0760	-0.0605 to 0.0617
z statistic	0.563	0.561	0.0201
Significance level	P=0.5737	P=0.5751	0.9840

whether antepartum, intrapartum, or post-partum. To predict the impact of perinatal asphyxia on severity of hypoxic ischemic insult, a variety of criteria have been used, such as APGAR score, which has been used since 1952. The accuracy of these criteria has been questioned because of low sensitivity and specificity. Neonatal acidemia has been associated with increased cerebral blood flow, which can enhance perfusion, thus, preventing hypoxic injury, but later leading to reperfusion injury, which is deleterious to the developing brain.[11] Acidemia due to permissive hypercarbia has been associated with decreased risk for hypoxic injury. However, acidosis due to hyperlactatemia is always associated with increased risk of brain damage.[12]

Our study confirmed a significant association between pH and subsequent development of HIE. Using a cut-off point of pH< 7.13, there was 100% sensitivity and 99.11% specificity for development of HIE stage II/III after perinatal asphyxia. Similar results were reported by a previous study on 250 neonates with acidemia.[13] They found newborn arterial pH of ≤ 7.1 had a strong association with hypoxic-ischemic encephalopathy, neonatal intensive care unit admissions, and a composite adverse outcome parameter. Other studies also reported results similar to our study.[14,15]

Extensive study is available on lactate associations with sequalae of perinatal asphyxia, but few studies could be found on predictive ability of lactate in HIE severity. We found that a lactate cut-off of >6.89 mmol/L had 100% sensitivity and 85.11% specificity for the evolution of perinatal asphyxia to HIE stage II/III. Similarly, a previous study derived a cut-off point of <7.1 mmol/L, with 48% sensitivity and 85% specificity.[16] For measurement of urinary lactate, creatinine ratio >1.0 was found to predict death or impairment, with positive and negative predictive values of 69% and 96%, respectively, in a selected group of neonates with perinatal asphyxia.[17]

Our study showed that base deficit also had a correlation with severity of HIE after birth asphyxia. The cut-off point of >7 mEq/L had 82.4% sensitivity and 91.76% specificity. This finding was in agreement with a study which derived a cut-off point of >7.5 mmol/l, with sensitivity of 94% and specificity of 67%.[16] A previous study found that at base deficit of >11 mmol/L, neonatal complications after perinatal asphyxia increased with 86% sensitivity and 79% specificity.[11] Our lower cut-off point could be because the specimen was from cord blood, while other studies used neonatal arterial blood, either at 1hr after birth or later.[14-16]

Comparing the predictive ability of all three parameters, we found no significant differences, thus, we conclude that all have similarly good predicative ability to determine the subsequent development of severity of HIE after perinatal asphyxia. This finding was in agreement with previous studies.[18-20]

The limitation of our study was the small sample size of study neonates. However, given the catchment area and total deliveries in our hospital and the lower incidence of perinatal asphyxia nowadays because of advanced neonatal science and personnel well-trained in neonatal resuscitation, keeping a confidence interval of 95% and margin of error at 5%, a sample size of 64 is acceptable for any study on perinatal asphyxia.

In conclusion, umbilical arterial blood pH, lactate, and base deficit all have good sensitivity and specificity for predicting severity of HIE in infants with perinatal asphyxia, and are equally effective in such predictions.

Conflict of Interest

None declared.

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Original Article

Platelet counts in epileptic children receiving valproic acid

Lilik Indrayati, Fadhilah Tia Nur, Bambang Soebagyo

Abstract

Background Epileptic seizures are a transient occurrence resulting from abnormal, excessive, or synchronous neural activity in the brain. Epilepsy requires long-term treatment, increasingly larger doses, and combination therapy. Anti-epileptic drugs (AEDs), especially valproic acid (VPA), are the main treatment of choice. Thrombocytopenia is the most common adverse event from AEDs.

Objective To evaluate platelet counts in epileptic children receiving valproic acid monotherapy vs. polytherapy.

Methods This analytic, observational, retrospective cohort study was conducted in children with epilepsy below 18 years of age and treated in Dr. Moewardi Hospital, Surakarta, Central Java. Subjects had received VPA treatment for at least 6 months, either as monotherapy or polytherapy. There were 40 subjects in each group (VPA monotherapy vs. VPA polytherapy). The exclusion criteria were patients who had thrombocytopenia and did not take valproic acid regularly. The data was taken from laboratory and the outcome assessed was decreasing of platelet count.

Results Administration of VPA as monotherapy *vs.* polytherapy was not significantly associated with incidence of thrombocytopenia. However, duration of VPA use > 2 years was associated with significantly greater proportion of thrombocytopenia, with OR 33.0 (95%CI 4.157 to 261.962; P=0.001) compared to VPA use < 2 years. Similarly, VPA dose of >30 mg/kg/day was significantly associated with greater proportion of thrombocytopenia, with OR 4.081 (95%CI 1.337 to 12.458; P=0.013) compared to <30 mg/kg/day dosage.

Conclusion Incidence of thrombocytopenia is not significantly different between VPA as a monotherapy and polytherapy. However, higher VPA dose and longer VPA duration are associated with higher proportion of thrombocytopenia. **[Paediatr Indones. 2020;60:13-7; doi: http://dx.doi.org/10.14238/pi60.1.2020.13-7]**.

Keywords: *valproic acid; monotherapy; polytherapy; platelets; epileptic; epilepsy*

Epileptic seizure is defined as a transient occurrence of signs and/or symptoms resulting from an abnormal, excessive, or synchronous neural activity in the brain.[1] Cipto Mangunkusumo Hospital, Jakarta reported that of 1,700 patients in 2009 and 2010, 218 generalized epilepsy and 71 focal epilepsy patients were newly-diagnosed annually.[2]

Epilepsy often requires a long period of treatment, progressively higher doses, and combined therapy. Adverse events of long-term anti-epileptic drugs (AEDs) include hepatotoxicity, as well as behavioral and memory disorders. They may also affect the hematologic and endocrine systems, bone density, and lipid profiles.[3]

Close hematologic monitoring must be done in patients using carbamazepine, phenytoin, and VPA. Thrombocytopenia is the most common side effect of AEDs, especially VPA. Its mechanism is

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unclear, though VPA is thought to have a direct toxic effect on bone marrow, as well as trigger the production of autoantibodies against platelets.[4] To our knowledge, no study to date has been conducted on thrombocytopenia incidence in children with epilepsy receiving long-term VPA in Dr. Moewardi Hospital, Surakarta. Therefore, we aimed to evaluate platelet counts in patients receiving either VPA monotherapy or polytherapy.

Methods

This retrospective, observational, cohort study was performed in Dr. Moewardi Hospital, Surakarta, Central Java, from July 2018 to January 2019. The study subjects were pediatric patients below 18 years of age receiving VPA monotherapy or VPA with other AEDs (polytherapy) for at least 6 months. Patients who did not take AEDs regularly, had underlying thrombocytopenia (i. e. idiopathic thrombocytopenic purpura/ITP, dengue haemorhagic fever/DHF) were excluded. Parents or guardians provided written informed consent. We assessed decreasing of platelet count and thrombocytopenia incidence.

The sampling technique using an unpaired numeric analysis led to an estimated minimum required number of 40 subjects for each group (monotherapy *vs.* polytherapy). Platelet counts were performed by the clinical laboratory using venous blood specimens. A platelet cut-off of <150,000/uL was used to classify patients as thrombocytopenic. Statistical analyses were unpaired T-test, Mann-Whitney, and Chi-square tests. Results with P values <0.05 were considered to be statistically significant. This study was approved by Ethics Committee of Health Studies, Dr. Moewardi Hospital/Universitas Sebelas Maret Medical School, Surakarta.

Results

There were 80 pediatric epileptic patients aged < 18 years who fulfilled our inclusion criteria. The basic characteristics of subjects were sex, age, and the length of VPA use. The majority of subjects were male in both groups. Mann Whitney test revealed no significant difference in the mean age of patients between the monotherapy and polytherapy groups. Length of VPA use was significantly shorter in the monotherapy group [30.33 (SD 18.38) months]than in the polytherapy group [52.28 (SD 41.45) months] (P=0.023). The characteristics of subjects are shown in **Table 1**.

Table 1. Subjects' characteristics

Characteristics	Valproic acid		P value
	Monotherapy (n=40)	Polytherapy (n=40)	
Sex, n (%)			0.818*
Male	24 (60.0)	25 (62.5)	
Female	16 (40.0)	15 (37.5)	
Mean age (SD), months	53.13 (35.67)	73.80 (49.70)	0.070#
Mean length of VPA use (SD), months	30.33 (18.32)	52.28 (41.45)	0.023#

Note: *Chi-square test, #Mann-Whitney test

The mean platelet count of the VAP monotherapy and polytherapy groups were 218.45 (SD 88.81) K/dL and 241.92 (SD 84.74) K/dL, respectively. Fifteen (37.5%) patients in the monotherapy group and 9 (22.5%) patients in the polytherapy group had platelet counts ≤150,000/dL. The incidences of thrombocytopenia in VPA monotherapy and polytherapy are shown in **Table 2**.

Table 2. Type of VPA therapy and platelet count mean by groups

Platelet count	Type of VPA therapy	
	Monotherapy (n=40)	Polytherapy (n=40)
≤150,000/µL (n=24)	15	9
>150,000/µL (n=56)	25	31
Mean (SD), k/dL	218.45 (88.81)	241.92 (84.74)

Further statistical analyses of possible associations between VPA monotherapy and polytherapy, length of VPA use, as well as VPA dose on thrombocytopenia were conducted. Chi-square test revealed no significant differences in the percentages of patients receiving either monotherapy or polytherapy between the platelet count groups (P=0.143). The length of VPA use significantly affected the decrease of platelet

count in which the administration of VAP for more than two years increased the incidence of the decline in platelet count (P<0.001). High dose of VAP was statistically significant affect the decrease of platelet count (P=0.01) (**Table 3**).

Table 3. Analysis of platelet count groups with type of VPA therapy, length of use, as well as dose

Variables	Platelet		P value
	≤150,000/µL (n=24)	>150,000/µL (n=56)	
Valproic acid, n			
Monotherapy	15	25	0.143
Polytherapy	9	31	
Length of use, n			
≤ 2 years	1	33	<0.001*
> 2 years	23	23	
Dose, n			
≤ 30 mg/kg/day	5	29	0.010*
>30 mg/kg/day	19	27	

*Chi-square test

Multivariate analysis demonstrated that patients who received VPA therapy for more than two years would have a 33 times higher risk of-having a platelet count of ≤150,000u/L compared to those who received the therapy for less than two years (OR 33.0; 95%CI 4.157 to 261.962; P=0.001). In addition, VPA dose of >30mg/kg BW/day increased the risk of platelet decline by 4.081 compared to a dose of ≤30mg/kg BW/day (OR 4.081; 95%CI 1.337 to 12.458; P=0.013) (**Table 4**).

Discussion

In our study, 37.5% patients receiving VPA monotherapy and 22.5% patients with polytherapy had platelet count ≤150,000/dL. Decrease in platelet count may be associated with VPA therapy, whether monotherapy or in combination with other AEDs and the length of VPA use. In our study, VPA was mostly commonly combined with carbamazepine. The length of VPA use was in line with epilepsy therapy protocol. Typically, only VPA is administered, but if its maximum dose does not prevent seizures then other AEDs would be added. Thus, patients receiving polytherapy use VPA longer than those in monotherapy.[5]

Chi-square test revealed no significant difference in thrombocytopenia between the monotherapy and polytherapy groups. However, we found a significant association between thrombocytopenia and length of VPA use. This difference was then analyzed further with multivariate analysis revealing an OR of 33.0 (95%CI 4.157 to 261.962; P=0.001). An estimated 6-33% of patients taking VPA experience thrombocytopenia.[6] Therefore, it can be considered that not all VPA use will couse thrombocytopenia. A study noted that thrombocytopenia occurs more frequently in patients receiving monotherapy.[9] Another previous study also reported that thrombocytopenia occurred in 26.4% of patient using VPA monotherapy and 15.8% of

Table 4. Multivariate analysis of platelet count groups with type of VPA therapy, length of use, and dose

Variables	Platelet		OR (95% CI)	P value
	≤150,000/µL (n=24)	>150,000/µL (n=56)		
Valproic acid, n				
Monotherapy	15	25	0.484 (0.182 to 1.289)	0.147
Polytherapy	9	31		
Length of use, n				
≤ 2 years	1	33	33.0 (4.157 to 261.962)	0.001*
> 2 years	23	23		
Dose, n			4.081 (1.337 to 12.458)	0.013*
≤ 30 mg/kg/day	5	29		
>30 mg/kg/day	19	27		

patients using VPA polytherapy.[11] Although the VPA mechanism of action is unclear, it suggested that carbamazepine, the most common drug used in polytherapy, can lower VPA level so that thrombocytopenia incidence decreases.[10,11]

We found that the VPA dose of >30 mg/kg BW/day had a 4-fold probability for platelet count ≤150,000/dL. This finding was in agreement with a 2014 study where 1/5 of children taking VPA monotherapy for more than 6 months at a dose of 30 mg/kg BW/day experienced decreased platelet count.[12] Other studies showed that decreased platelet count was related to VPA dose and depended on the serum level of VPA (>100 mg/L).[12,13] However, we did not check serum VPA level, so we could not evaluate for its association to lower platelet count in our subjects.

In our study, the multivariate analysis revealed that the use of VPA for two years or less reduced thrombocytopenia incidence significantly. Type of VPA, whether monotherapy or polytherapy, was not associated with platelet count but increased length of VPA use correlated with thrombocytopenia. Generally, long-term VPA therapy in children with epilepsy leads to hematologic system disorders, one of which is thrombocytopenia.[6]

Our study had several limitations. The blood specimen collection was done randomly, so we could not evaluate for a trend in platelet count decrease. Also, we did not include subjects with similar length of VPA use, thus we could not accurately assess for differences of platelet count decline. In conclusion, there is no difference in incidence of thrombocytopenia in children with epilepsy receiving either valproic acid as monotherapy compared to polytherapy. However, significantly more subjects with longer VPA use and higher VPA dose are in the low platelet group.

Conflict of interest

None declared.

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Original Article

Detecting neurodevelopmental problems using the simple parent-reported screening tool in combination with primitive reflex assessment

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Abstract

Background *The Kuesioner Pra Skrining Perkembangan/KPSP (Developmental Pre-screening Questionnaire/*DPsQ) is a series of questions and instructions used as a developmental screening tool for children aged 3 months to 6 years. However, the DPsQ cannot fully detect the soft signs of future neurological disorders. However, the retained primitive reflex assessment as an adjunct to the DPsQ may be useful for such detection.

Objective To determine whether assessing for retained primitive reflexes can add to the usefulness of DPsQ as a neurodevelopmental screen in children aged 1 to 5 years.

Methods This cross-sectional study included children aged 1-5 years. Developmental screening was done using the DPsQ and retained primitive reflex assessment was performed using the Institute for Neuro-Physiological Psychology (INPP) screening and scoring guideline.

Results Of 46 subjects, 56.8% of children with normal DPsQ scores had not retained primitive reflexes, while 88.9% of children with suspect DPsQ score had retained primitive reflexes. Hence, children with suspect DPsQ score had a 10.5 times higher chance of retaining primitive reflexes (OR 10.50; 95%CI 1.19 to 92.73; P=0.034). Furthermore, 66.7-77.8% of children with suspect DPsQ score had retained the Moro reflex, asymmetrical tonic neck reflex (ATNR), and symmetrical tonic neck reflex (STNR). Neither gender nor age were significantly associated with either suspect DPsQ score or the presence of retained primitive reflexes.

Conclusion The DPsQ results correlate to integration of primitive reflexes, with 10.50 greater odds of children with 'suspect' DPsQ scores to have retained primitive reflexes. As such, retained primitive reflexes is not useful as a primary screen for future neurological problems. However, a high percentage of children (43.2%) with normal DPsQ scores also have retained primitive reflexes. **[Paediatr Indones. 2020;60:31-6; doi: http://dx.doi.org/10.14238/pi60.1.2020.31-6]**.

Keywords: *KPSP; DPSQ; retained primitive reflex; neurodevelopmental problems; children 1-5 years old*

The *Kuesioner Pra Skrining Perkembangan/KPSP* (*Developmental Pre-screening Questionnaire/*DPsQ) is a series of questions and instructions used as a developmental screening method for children aged 3 months to 6 years.[1] The DPsQ consists of 10 questions to be answered 'yes' or 'no' by parents. The DPsQ can be done in approximately 10-15 minutes. A DPsQ score of 9 or 10 indicates that the child has no impairment, while a score below 9 means that the child is suspected of having a developmental problem. Dhamayanti compared the Denver II Screening method as the gold standard to the DPsQ. Denver II Screening has high reliability (test-retest reliability=0.90 and interrater reliability=0.99). The sensitivity and specificity of DPsQ compared to Denver II were 60% and 92%, respectively.[1] A good developmental screen should have >70-80% sensitivity and specificity, which was not met by the DPsQ. With 60% sensitivity, 40% of

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children with developmental problems might not be detected by the DPsQ.[1] Therefore, in order to boost the sensitivity and specificity of DPsQ, especially in terms of detecting the soft signs of neurology disorders, we aimed to evaluate use of the retained primitive reflex assessment in combination with the DPsQ.

Primitive reflexes are automatic behavioral motor responses found early in life, which are subsequently inhibited and integrated.[2] Primitive reflexes are regarded as the training tool to develop voluntary movement as well as the development of many areas of the brain. As such, these reflexes are crucial in determining one's future neurodevelopment. Newborns, infants, and children react to stimuli with their primitive reflexes, either by moving toward or away from the stimulus. The development of these reflexes can determine the development of their motor system, which influences their rooting, sitting, lying down, crawling, grasping, walking, and many other movements.[3-5] There are four phases of development (1) the reflexive movement phase, (2) the rudimentary movement phase, (3) the fundamental movement phase, and (4) the specialized movement phase.[3,5]

In some cases, primitive reflexes are not inhibited nor integrated. This phenomenon may occur organicly, by a neural disruption. Most often the disruption can be found in the frontal lobe of the brain, which also consists of the motor cortex. However, other studies found that primitive reflexes may have an unknown cause. This problem may arise due to the lack of external stimuli that is needed to support normal development of children. The presence of primitive reflexes can also be one of the easiest, fastest, and most specific indicators of future neurodevelopmental problems, such as attention-deficit/hyperactivity disorder (ADHD), autism, mental retardation, learning problems, and cerebral palsy.[2,4,6–8]

Arditi noted that primitive reflexes were found in 20.2% of healthy children without ADHD, and 79.8% children with ADHD.3 There are numerous types of primitive reflexes in children, including rooting, sucking, grasping, and nucocephalic reflexes. Moreover, some studies found that the five primitive reflexes: Moro, spinal galant, symmetrical tonic neck reflex (SNTR), assymetrical tonic neck reflex (ATNR), and tonic labyrinthine reflex (TLR), were closely related with one's future learning capabilities, motor development, and neurodevelopment.[9-12]

Screening for retained primitive reflexes is crucial in determining the chance of future neurodevelopmental problems, as immediate intervention and treatment can be done. Unfortunately, there is no adequate data in the assessment of retained primitive reflexes in healthy children. This study aimed to determine whether assessing for retained primitive reflexes can add to the usefulness of DPsQ as a neurodevelopmental screen in children aged 1 to 5 years.

Methods

This cross-sectional analytical study included children aged 1-5 years in Pancoran Mas, Kecamatan Lio, Depok, West Java, Indonesia and 2 villages in Bogor (Kampung Barongsang and Kampung Tapos).

Prior to the study, ethics was reviewed and approved by Universitas Indonesia Medical School/ Dr. Cipto Mangunkusumo Hospital Research Ethical Committee. Approval from the heads of the villages was attained before the assessment is conducted. Informed consent was obtained from subjects' parents/ guardians.

Data were collected from 46 healthy children in Pancoran Mas, Depok on August 11, 2018 and in Bogor on May 29 and May 31, 2019. Subjects were obtained from the local *pendidikan anak usia dini*/ PAUD (early childhood education), the *pos pelayanan perpadu/posyandu* (integrated healthcare service), and by door-to-door survey method. Subjects will be included in the study if they fulfilled all eligbility criteria. Inclusion criteria include (1) children aged 1-5 years old and (2) accompanied by subjects' parents/ guardians. Exclusion criteria include (1) children were currently sick on the assessment day and (2) whose parents/guardians refused for the child to be screened.

Subjects underwent two assessments, the DPsQ development screening and the retained primitive reflex assessment. The DPsQ had a set of questions that were answered by parents/guardians, as well as particular instructions for the child to follow. The child's development status was defined based on scoring standards, normal for scores of 9-10 and suspect for scores 0-8.[13] The retained primitive reflex assessment was conducted by physical examination. The child performed certain maneuvers as instructed

by the examiner. If not possible, the examiner would help the child to do the maneuvers. The assessments were done using the *Institute for Neuro Physiological Psychology* (INPP) screening test.[4]

Stastistical analysis was done using *SPSS for Mac OS version 24* using primary data obtained from DPsQ and retained primitive reflex assessments. Data analysis consisted of univariate analysis, categorical data displayed in tables to show frequency and percentage, as well as bivariate analysis, to evaluate for possible associations between the independent variable and dependent variables. Categorical variables were evaluated by Chi-square test. Non-normally distributed data was analyzed by Fischer's exact test. Results with P values <0.05 were considered to be statistically significant. Odds ratio (OR) and 95% confidence interval (CI) are indicative of the strength of association.

Results

Forty-six healthy children, 24 males and 22 females, met the inclusion criteria. The demographic characteristics of subjects are shown in **Table 1**. Of the 46 subjects, 37 children scored 9 or 10 on the DPsQ (80.4%) and 9 children scored less than 9 (19.6%). In addition, 24 children had observable retained primitive reflexes (52.2%), as follows: Moro (12 subjects; 26.1%), spinal galant (3 subjects; 6.5%), ATNR (14 subjects; 30.4%), STNR (17 subjects; 37.0%) and TLR (0 subjects; 0%).

Table 1. Demographic characteristic of subjects (N=46)

Characteristics		n(%)
Age, years	1-2	16 (34.8)
	2-3	11 (23.9)
	3-4	10 (21.7)
	4-5	9 (19.6)
Gender	Male	24 (52.5)
	Female	22 (47.8)
DPsQ score	Normal	37 (80.4)
	Suspect	9 (19.6)
Primitive reflexes	Moro	12 (26.1)
	Spinal galant	3 (6.5)
	ATNR	14 (30.4)
	STNR	17 (37.0)
	TLR	0 (0)

ATNR=asymmetrical tonic neck reflex, STNR= symmetrical tonic neck reflex, TNR=tonic labyrinthine reflex

The most prevalent age group in which children scored suspect on DPsQ was 3 to 4-yearsold (5/10 children) and the least prevalent was 1 to 2-years-old (1/16 children). There were 4/24 boys and 5/22 girls who scored less than 9 in DPsQ assessment. However, there was no significant correlation between gender and suspect DPsQ score (P=0.18). Nor were there significant correlations between age or gender and the presence of retained primitive reflexes (P=0.758), AS shown in **Table 2**.

As shown in **Table 3**, suspect DPsQ score was significantly associated with the presence of primitive reflexes, with 8/24 of suspect DPsQ subjects having retained primitive reflexes (OR 10.50; 95%CI 1.19 to 92.73; P=0.034) (**Table 3**).

Table 4 shows the correlations between the individual primitive reflex categories and DPsQ scores. Children with suspect DPsQ scores were more likely to have retained primitive reflexes than children with normal DPsQ score. The most common primitive reflex found in children with normal DPsQ score was STNR (10/37), while in children with suspect DPsQ score, the percentage of subjects with STNR and Moro reflexes were equal (7/9). Spinal galant was more frequently found in children with normal DPsQ score, however,

Table 2. Prevalence of retained primitive reflexes with age groups and gender (N=46)

Variables	Primitive reflexes		P value
	Not present (n=24)	Present (n=22)	
Age, n			
1-2 years	7	9	
2-3 years	7	4	
3-4 years	4	6	
4-5 years	6	3	
Gender, n			
Male	12	12	0.758*
Female	10	12	

*Chi-square

Table 3. Retained primitive reflexes in children with normal and suspect DPsQ score (N=46)

Primitive reflexes	DPsQ		OR (95%CI)	P value
	Suspect	Normal		
Present (n=24)	8	16	10.50 (1.19 to 92.73)	0.034*
Not present (n=22)	1	21		

*Fisher's exact test

the difference was not significant. Since none of the subjects had TLR, we could not evaluate it.

Table 4. Distribution of retained primitive reflexes in children with normal and suspect DPsQ score (N= 46)

Primitive reflexes	DPsQ		P value*
	Normal (n=37)	Suspect (n=9)	
Moro	5	7	0.000
Spinal galant	3	0	1.000
ATNR	8	6	0.039
STNR	10	7	0.008
TLR	0	0	Invalid

*Fisher's exact test

Discussion

Studies on primitive reflexes have been widely conducted on children with cerebral palsy and ADHD.[3,14-15] However, there are few studies about retained primitive reflexes in healthy children. One such study in Poland on 35 healthy preschool children aged 4 to 6, was quite similar to our research. The mean age of subjects in their study was 4.7 years, while our study of 46 children had a mean age of 2.9 years old. They assessed for only 3 types of retained primitive reflexes (ATNR, STNR and TLR), while we assessed for 5 types (ATNR, STNR, TLR, Moro and spinal galant). They reported that 87% of boys and 89% of girls had at least one persistent primitive reflex of varying degrees. In contrast, we found that only 50% of boys and 54.5% of girls had persistent primitive reflexes.[15] Some possible reasons for the difference in the frequency are different biological and intrapersonal factors that affect the integration of primitive reflexes in child development and that the examination and scoring results are dependent on the examiner's skill and bias.[16]

In our DPsQ assessment, 37 children scored 9-10, 5 children scored 7-8, and 1 child scored below 7. Subjects were categorized into either the normal group (score 9-10) or the suspect group (score <9).[1] The independent variables of age and gender had no significant correlation with DPsQ scores. An exception was the 3 to 4-years-age group, in which 50% of subjects had suspect DPsQ scores, while the percentages of suspect DPsQ scores in the other age groups were much lower, ranging from 6.3-11.1%.

In our 46 healthy subjects, children with low DPsQ scores had a tendency to have retained primitive reflexes (8/9). Moreover, children with high DPsQ scores had a slightly higher tendency to have integrated primitive reflexes (21/37).

Each retained primitive reflex has certain implications for future development of the child, with potential effects on concentration, attention, athletic capability, and other aspects.[3] If the Moro reflex is not integrated, it will negatively impact concentration and focus, due to the accumulation of stimuli in the brain stem, and may manifest as hyperactivity and hypersensitivity. A retained Moro reflex can also be a very early sign of anxiety in the future.[3,17] Furthermore, a retained spinal galant reflex can affect athletic capability, lead to an unbalanced gait, increase the risk of scolisosis, and decrease concentration, since small spinal stimuli may disturb focus and make the individual uncomfortable.[3,18] Integrated ATNR is a sign of balanced coordination between the left and right sides. If ATNR persists, it could be a soft sign of poor hand-eye coordination affecting balance, in general, and concentration.[3,17] The STNR regulates the balance between the upper and lower body. If it is not well integrated, a child will be unable to fully control the hand and leg while seated or when the head is flexed and extended, leading to poor focus and concentration. Lastly, retained TLR mainly leads to disturbances in balance and coordination, because flexion and extension stimulate muscle contraction and relaxation.[3,14-15]

Primitive reflexes play a huge developmental role, assisting neonates to perform important movements. They are gradually integrated as the child develops. Mature response and integrated primitive reflexes show that the central nervous system has matured. This process consists of transitioning control from the brain stem to a more voluntary movement controlled by the brain cortex.[9,16,19] The assessment of retained primitive reflexes serves as a screening tool to detect possible soft signs of future neurological problems. In comparison, the DPsQ is a diagnostic tool to assess the child development. With "Yes" or "No" answers on on 9 to 10 questions for each age group, this instrument can be used to evaluate the development of the child. It is used widely by many healthcare facilitators in Indonesia. We found that subjects with suspect DPsQ scores had a high likelihood of having retained primitive reflexes (8/9 of subjects). However,

the absent of integrated primitive also correlated with a higher score of DPsQ (16/37). And based on statistical tool of Fisher's exact test, the P value is 0.034, which signifies that it is significant. Out of all samples, DPsQ can detect 7/12 persistent Moro reflex, 6/14 persistent ATNR and 7/17 STNR.

We found that of 9 healthy children with suspect DPsQ score, 7 had retained Moro reflex, 7 had STNR, and 6 had ATNR. On the other hand, of 37 healthy children with normal DPsQ score, the percentages of children with retained primitive reflexes were significantly lower, as 5/37 had retained Moro reflex, 10/37 had STNR, and 21.6% 8/37 ATNR. These data suggest that both assessments, DPsQ and INPP retained primitive reflex, can successfully be used to assess brain maturity. Such assessment of milestones is crucial to ensure the best possible development. Brain maturity is influenced by many factors and will be achieved faster if there are external stimuli from parents or caregivers and interpersonal relationships with people in their surroundings. Delayed brain maturity can lead to developmental problems, including autism, cognitive problems, mental retardation, ADHD, and cerebral palsy. A previous study found that 15% of school-age children had mild neurodevelopmental problems, mostly in coordination, fine motor skills, as well as decreased muscle tone.[20]

The analysis of retained spinal galant and TLR were not significant and invalid, respectively. The small sample size of only 3 healthy children (8.1%) with retained spinal galant reflex likely led to a lack of significance. Since none of our subjects had retained TLR, this reflex could not be evaluated. These two reflexes may have been lacking/absent because they are typically the first two reflexes to be integrated, at approximately 3-4 months old for TLR and 3-6 months for spinal galant. Other factors may include external stimuli, adequate movement, and adequate exercise.[3-4] A larger sample size is needed for more reliable results.

There were 16 healthy children with normal DPsQ score (43.2%) and at least one retained primitive reflex. Even though the DPsQ score proportionally correlated with the integration of primitive reflexes, some children still had retained primitive reflexes. Hence, retained primitive reflexes is useful for further screen, especially to screen future soft sign neurological problems.This research had several limitations, such as not evaluating other independent variables, such as the child's behavior at school and at home, parental education, history of birth, or nutritional status, which may correlate to retained primitive reflexes and DPsQ score. Further study on the effect of suspect DPsQ score and late integration of primitive reflexes on school-aged children could also be done.

In conclusion, there was a significant association between low DPsQ scores and the presence of retained primitive reflex, and there were 10.50-higher odds of children with suspect DPsQ score to have retained primitive reflexes. The DPsQ can detect 41.2-58.3% of 3 primitive reflexes successfully assessed.

For further research, other independent variables that may affect DPsQ score and integration of primitive reflexes may also be assessed, such as parental education level, paternal occupation, birth history, attachment, cigarette exposure, and familial socioeconomic level.

Community and healthcare providers should assess for retained primitive reflexes in children more than 1 year old, especially in preschool-aged children, since retention can likely affect their future development. Parents can also assess their children for retained primitive reflexes to detect possible soft signs of neurological problems, so that early intervention can be done. Parents should be encouraged to increase the intensity of their child's exercise level, as well as provide appropriate attachment and external stimuli to induce the integration of primitive reflexes.

Conflict of Interest

None declared.

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Original Article

Mid-upper arm circumference measurement for severe malnutrition screening in underfives

Stephanie Adelia, Johannes Capistrano Susanto

Abstract

Background Severe malnutrition in Indonesia remains abundant. Severe malnutrition has been assessed by several methods, including mid-upper arm circumference (MUAC) and weight-for-height z-score (WHZ). As a screening method, MUAC is expected to be useful for identifying malnutrition in communities.

Objective To determine whether MUAC measurements can be used for screening severe malnutrition at the community level in Indonesia.

Method A cross-sectional study was conducted in 853 children aged 6-59 months who came to an integrated health service post (Posyandu) in Semarang, Central Java. Anthropometric measurements were performed by cadres and researchers and included MUAC and WHZ. Statistical analysis was done by McNemar test; results with P values >0.05 indicated no significant difference. Sensitivity and specificity were determined by 2 x 2 tables. The MUAC cut-off values were determined by receiver-operating characteristic (ROC) curve.

Results Eight hundred fifty-three out of 1,115 children met the inclusion criteria, consisting of 419 (49.1%) boys, with most over the age of 2 years (57.2%). Kappa test revealed good inter-rater reliability in measurements between the cadre and researchers (Kappa=0.726). There were significant differences between MUAC (by cadres) and below red line status as well as WHZ, between MUAC (by researchers) and WHZ, as well as MUAC (by cadre and researchers) with WHZ and height-for-age z-score/HAZ . Sensitivity, specificity, PPV, and NPV of MUAC (by cadre) were 12.5%, 99.9%, 75%, and 97.5%, respectively, while those by the researchers were 16.7%, 99.6%, 57%, and 97.6%, respectively. In this study, MUAC of 14 cm was the best cut-off for severe malnutrition.

Conclusion The MUAC measurement of 14 cm can be used for screening severe malnutrition in underfives at community. **[Paediatr Indones. 2020;60:42-52; doi: http://dx.doi.org/10.14238/pi60.1.2020.42-52].**

Keywords: *mid-upper arm circumference; WHZ; severe malnutrition; underfives*

In Indonesia, severe malnutrition is abundant and often accompanied by various complications.[1] These complications can either be short-term such as increased morbidity, mortality, and disability, or long-term such as decreased intellectual ability, economic productivity, reproductive function, short stature, and metabolic and cardiovascular diseases. The lack of an easy, inexpensive, and widely available screening tool for severe malnutrition contributes to its abundance.[2] Furthermore, the integrated health service posts (*pos pelayanan terpadu/posyandu*) have not optimally performed and promoted growth monitoring.[3]

Current practice in the *posyandu* is to assess only children who fall below the red line (*bawah garis merah* or BGM), which is categorized as malnourished. Such children should be reported to the community health centre (*pusat kesehatan masyarakat/puskesmas*). However, children with BGM might not be severely malnourished, and vice versa; children with severe

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acute malnutrition might not have yet reached BGM status.[3-5] Hence, the BGM designation can be misleading.

Screening for severe acute malnutrition in Indonesia has not been optimally carried out because the criteria for severe malnutrition issued by the Ministry of Health is based on the weight-for-age indicator. Weight-for-age represents only body weight related to age, without being able to depict a process of acute malnutrition, allowing for 'wasting' and 'stunting' to not be identified. Acute malnutrition can be most accurately assessed by how thin someone is, thus the anthropometrical indicators fitting for this purpose would be MUAC and WHZ.[4-7]

In Africa and south Asia, MUAC has been used as a screening method for under-fives in order to detect malnutrition cases early, for optimal intervention. The MUAC measurements can be done by field workers, such as posyandu cadres, since it is easy to perform and has high accuracy, reliability, sensitivity, and specificity.[8-10] We aimed to determine if MUAC can be useful for screening severe malnutrition in underfives at the community level.

Methods

This analytical cross-sectional study was done on 853 children aged 6-59 months who came to the posyandus in Semarang, Central Java, Indonesia. The inclusion criteria were 6-59 months of age and parental consent. The exclusion criteria were children who suffered from congenital or chronic diseases that are known to cause malnutrition, certain syndromes, edema, or organ enlargement (organomegaly). The cadres collected subjects' MUAC and body weight data. The results were documented and plotted to subjects' growth charts (*Kartu Menuju Sehat/KMS*). The MUAC, body weight, height or length, and the assessment of WHZ, WAZ, and HAZ were done by the researchers.

Body weight, body length/height, and MUAC measured by cadres and researchers; each measurement repeated 3 times and then we took the average. Body weight measured with *Onemed* digital weight scale. Body weight status was classified according to *WHO Child Growth Standard* (weight for age): severely underweight if < -3 standard deviations (SD) of the median, underweight if < –2 SD of the median, normal weight if > -2 SD and < 2 SD of the median, and overweight if > +2 SD of the median. Nutritional status was classified according to *WHO Child Growth Standard* (weight for length/height): severe malnutrition if < -3 SD of the median, malnutrition if < –2 SD of the median, well nourished if > -2 SD and < 2 SD of the median, overweight if > +2 SD of the median, and obesity if +3 SD of the median. Body length measured with *Onemed* infantometer while height measured with GEA stature meter. Stature was classified referring to *WHO Child Growth Standard* (length/height for age), as followed: very short stature if < –2 SD of the median, short stature if < –2 SD of the median, normal if > -2 SD and < 2 SD of the median, and tall if > 2 SD of the median. Measurement of MUAC used WHO MUAC tape, by measuring the middle part of the upper arm and the result was expressed in color: red if MUA ≤11.5 cm, not red if MUA > 11.5 cm.

Based on WHO 2013 recommendation, severe acute malnutrition in children aged 6-59 months is defined by MUAC ≤ 115 mm or WHZ ≤ -3 SD with HAZ > -2 SD, while severe chronic malnutrition is diagnosed for MUAC ≤ 115 mm or WHZ ≤ -3 SD with HAZ ≤ -2 SD. However, Ministry Health of Indonesia still used birth weight for age indicator to describe severe malnutrition that also called *bawah garis merah* (*BGM*), as the body weight was under the red line of the grow charts (KMS). The reliability of MUAC measurement between cadres and researcher were examined by Kappa test. This MUAC measurement with cut off 11.5 cm was compared to WHZ by McNemar test for the identification of severe malnutrition cases. Sensitivity and specificity of MUAC were calculated using 2x2 tables. The optimal cut-off point for MUAC was obtained by ROC curve. This study was approved by the Medical Research Ethics Commission at Diponegoro University Medical School/Dr. Kariadi Hospital, Semarang.

Results

A cross-sectional study was conducted in Semarang in April 2018 in children aged 6-59 months who visited the *posyandus* that was selected for sampling. Semarang has 37 main *puskesmas* scattered in 16

sub-districts. In cluster sampling, 5 *puskesmas* were selected. Additional sampling was done to determine the selection of the *posyandu*, resulting in 30 *posyandus* fostered by said *puskesmas*. In total, 1,115 children visited the selected posyandu, but 98 children were less than 6 months of age and 64 children had incomplete data since they were uncooperative during the measurement, thus 853 children met the inclusion criteria (**Figure 1**). The characteristics of the study participants are shown in **Table 1**.

Table 1 shows that the subjects consisted of 419 boys (49.1%) and 434 girls (50.9%), with the majority of subjects older than 2 years of age (57.2%). The subjects' mean MUAC was 14.4 (SD 1.4) cm for children <1 year of age, 14.9 (SD 1.6) cm for children aged 1-2 years, and 16.4 (SD 2.2) cm in children aged >2 years. The subjects' mean WHZ was -0.47 (SD 1.25) for children <1 year of age, -0.48 (SD 1.36) for children aged 1-2 years, and -0.22 (SD 1.76) in children aged >2 years (**Table 2**).

The MUAC measurements were done by the cadres and the researchers. Inter-rater reliability was

Table 1. Basic characteristics of participants

Characteristics	N=853
Gender, n (%)	
Male	419 (49.1)
Female	434 (50.9)
Age, n (%)	
<1 year	108 (12.7)
1-2 years	257 (30.1)
>2 years	488 (57.2)
Body weight status, n (%)	
Severely underweight	27 (3.2)
Underweight	85 (10)
Normal	13 (83.6)
Overweight	28 (3.2)
Nutritional status, n (%)	
Severe malnutrition	24 (2.8)
Malnutrition	71 (8.3)
Well-nourished	730 (85.6)
Overweight	17 (2.0)
Obesity	11 (1.3)
Stature, n (%)	
Very short stature	27 (3.2)
Short stature	106 (12.4)
Normal	706 (82.8)
Tall	14 (1.6)

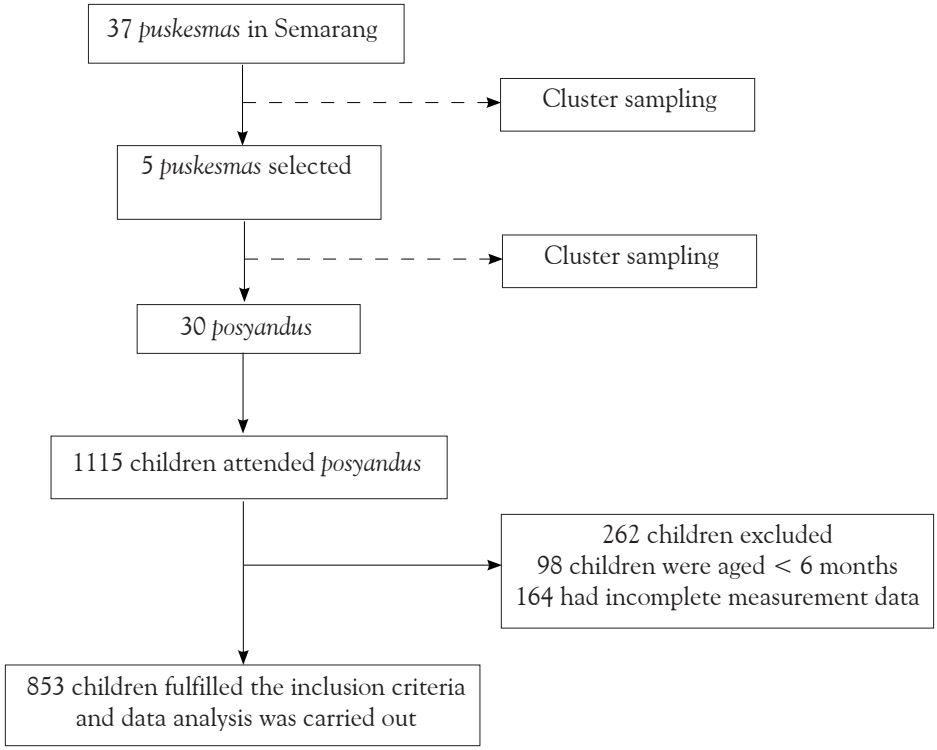

Figure 1. Study participant flow-

Table 2. Mean MUAC and WHZ based on age and sex

Age group	MUAC		WHZ (SD)	
	n	Mean (SD)	n	Mean (SD)
<1 year	108	14.4 (1.4)	108	-0.47 (1.25)
1-2 years	257	14.4 (1.6)	257	-0.48 (1.36)
>2 years	488	16.4 (2.2)	488	-0.02 (1.76)

analyzed by Kappa test, as shown in **Table 3**.

Table 4 shows the analyses of MUAC measurements performed by the cadres compared to (A.) WHZ, (B.) BGM, and (C.) Z-score (WHZ and HAZ). Severe acute malnutrition screening was defined as WHZ ≤ -3 SD and HAZ > -2 SD, and severe chronic malnutrition was defined as WHZ ≤ -3 SD and HAZ ≤ -2 SD. McNemar analysis revealed significant differences between MUAC and WHZ, MUAC and BGM, and MUAC and WHZ/HAZ for both acute and chronic severe malnutrition screening.

Table 5 shows the analysis of MUAC performed by researcher compared to (A.) WHZ, (B.) Z-score to evaluate if MUAC measurement could be utilized for severe acute malnutrition screening (WHZ ≤ -3 SD, HAZ > -2 SD), and severe chronic malnutrition (WHZ ≤ -3 SD, HAZ ≤ -2 SD). McNemar test revealed that MUAC measurement (by researcher)

Table 3. Inter-rater reliability of MUAC measurements by cadres and researchers (Kappa test)

		MUAC (researchers)		Total	Kappa value	P value
		≤11.5 cm	>11.5 cm			
MUAC (cadres)	≤11.5 cm	4	0	4	0.726	0.000
	>11.5 cm	3	846	849		
Total		7	846	853		

Table 4. Comparison of MUAC (by the cadres) and:
A. WHZ

		MUAC (cadres)		Total	P value
		≤11.5 cm	>11.5 cm		
WHZ	≤-3 SD	3	21	24	0.000
	> -3 SD	1	828	829	
Total		4	849	853	

McNemar test

B. *BGM*

		MUAC (cadres)		Total	P value
		≤11.5 cm	>11.5 cm		
BGM status	*BGM*	1	15	16	0.008
	Non-*BGM*	3	834	837	
Total		4	849	853	

McNemar test

C. WHZ and HAZ

		MUAC (cadres)		Total	P value
		≤11.5 cm	>11.5 cm		
MUAC (cadres)	≤11.5 cm	3	0	3	0.000
	> 11.5 cm	20	1	21	
Total		23	1	24	

McNemar test

Table 5. Comparison of MUAC (by researchers) and:

A. WHZ

		MUAC (researchers)		Total	P value
		≤11.5 cm	>11.5 cm		
WHZ	≤-3 SD	4	20	24	0.000
	> -3 SD	3	826	829	
Total		7	846	853	

McNemar test

B. WHZ and HAZ

		Severe malnutrition		Total	P value
		Acute	Chronic		
MUAC (cadres)	≤11.5 cm	4	0	4	0.000
	> 11.5 cm	19	1	20	
Total		23	1	24	

McNemar test

Table 6. Sensitivity, specificity, positive predictive value, negative predictive value for MUAC compared to WHZ

A. MUAC cut-off of 11.5 cm

		WHZ		Total
		≤-3 SD	>-3 SD	
MUAC (cadres)	≤11.5 cm	3	1	4
	> 11.5 cm	21	828	849
Total		24	829	853

		WHZ		Total
		≤-3 SD	>-3 SD	
MUAC (researchers)	≤11.5 cm	4	3	7
	> 11.5 cm	20	826	846
Total		24	829	853

Variables	MUAC (cadres)	MUAC (researchers)
Sensitivity (%)	12.5	16.7
Specificity (%)	99.9	99.6
Positive predictive value (%)	75.0	57.1
Negative predictive value (%)	97.5	97.6

B. MUAC cut-off of 13.8 cm

		WHZ		Total
		≤-3 SD	>-3 SD	
MUAC (researchers)	≤13.8 cm	22	107	129
	> 13.8 cm	2	722	724
Total		24	829	853

Variables	MUAC (researchers)
Sensitivity (%)	91.7
Specificity (%)	87.1
Positive predictive value (%)	17.1
Negative predictive value (%)	99.7

C. MUAC cut-off of 14 cm

		WHZ		Total
		≤-3 SD	>-3 SD	
MUAC (researchers)	≤14 cm	23	163	186
	> 14 cm	1	666	667
Total		24	829	853

Variables	MUAC (researchers)
Sensitivity (%)	95.8
Specificity (%)	80.3
Positive predictive value (%)	12.4
Negative predictive value (%)	99.9

D. MUAC cut-off of 15.8 cm

		WHZ		Total
		≤-3 SD	>-3 SD	
MUAC (researchers)	≤15.8 cm	24	495	519
	> 15.8 cm	0	334	334
Total		24	829	853

Variables	MUAC (researchers)
Sensitivity (%)	100
Specificity (%)	40.3
Positive predictive value (%)	4.6
Negative predictive value (%)	100

was significantly different from WHZ in severe malnutrition screening. In addition, MUAC was significantly different from WHZ and HAZ for both acute and chronic severe malnutrition screening.

In 2013, the WHO recommended using WHZ ≤ -3 SD to diagnose severe malnutrition. As such, we compared MUAC to WHZ as the gold standard, to determine sensitivity, specificity, positive predictive value, and negative predictive value of various MUAC cut-off points [(A.) 11.5 cm, by cadres and researchers; (B.) 13.8 cm by researchers; (C). 14 cm by researchers; and (D.) 15.8 cm by researchers]. From the ROC curve, WHZ ≤ -3 SD area under curve value was 0.926 (between 0.878 and 0.974), fitting the MUAC measurements of 13.8 cm, 14 cm, and 15.8 cm. Using a 2x2 table, we determined sensitivity, specificity, positive predictive value, and negative predictive value from several MUAC points (**Table 6**).

In our study, the ROC curve showed that MUAC sensitivity and specificity for severe malnutrition screening could be optimized if the cut-off point was increased. The cut-off point of 14 cm was selected because it had 95.8% sensitivity, 80.3% specificity, 12.4% positive predictive value, and 99.9% negative predictive value, thus, it could yield better results if used for severe malnutrition screening in the community (**Figure 2**).

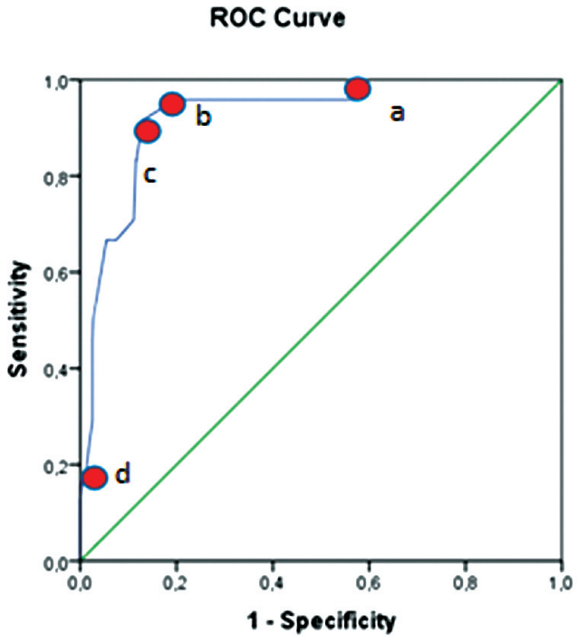

Figure 2. The ROC curve for sensitivity and specificity for MUAC compared to WHZ ≤-3 SD in severe malnutrition screening (red dots=sensitivity and specificity from several MUA cut off points: a. 15.8 cm, b. 14 cm, c. 13.8 cm, d. 11.5 cm)

Discussion

Mid-upper arm circumference (MUAC) and WHZ anthropometry indexes are used to describe a child's level of emaciation. The emaciation level indicating a severe process of weight loss, where this condition is associated with acute malnutrition condition.[11] Our study examined the appropriateness of MUAC measurements for screening severe malnutrition in the community.

This study was conducted on 853 subjects aged 6-59 months, with more females (50.9%) than males (49.1%). Most subjects were aged> 2 years (57.2%), and most subjects had well-nourished (85.6%) nutritional status. Of the 853 subjects, MUAC ≤ 11.5 cm was found in 4 children (0.5%) based on measurements by the cadres, and 7 children (0.8%) based on measurements by researchers. Although different percentages were obtained between the cadres and researchers, Kappa test revealed good inter-rater reliability coefficient values of 0.726 and P=0.000.

We also conducted a comparison test between MUAC and bawah garis merah (*BGM*/ below the red line), weight/age ≤ -3 SD) because the interpretation of the measurement results is mainly based on the *BGM* at the *posyandu*. Children with *BGM* status are considered to have malnutrition and referred to the *puskesmas*. However, reporting to the *puskesmas* does not necessarily mean that the children will be directly followed up unless they already have complications, because the *puskesmas* does not have confirmation of the child's nutritional status, including weight/ height (WHZ). In fact, children with *BGM* may not necessarily even have severe acute malnutrition and vice versa, while children with severe acute malnutrition may not have *BGM* status.[3-5]

There was a significant difference between MUAC and *BGM* for diagnosing severe malnutrition. This finding was in agreement with a previous study

that the weight/age indicator only reflects body mass for age, so it cannot be used as a diagnostic criteria for acute malnutrition, which requires prompt management.[12] Cases of severe acute malnutrition in Indonesia cannot be optimally treated, because screening with BGM criteria identifies children with chronic malnutrition, not acute malnutrition.[4,7]

In our study, we also compared MUAC to Z score (WHZ ≤ -3 SD) for the diagnosis of severe malnutrition. McNemar test revealed a significant difference between MUAC measurement and WHZ ≤ -3 SD (by both cadres and researchers). In addition, MUAC measurement and WHZ/HAZ to assess acute vs. chronic severe malnutrition was also significantly different. Hence, we can conclude that MUAC <11.5cm cannot be used as a single anthropometric indicator for diagnosing severe malnutrition, either acute or chronic, due to the incompatibility between MUAC and WHZ ≤ -3 SD, which is the gold standard for diagnosing severe malnutrition anthropometrically. We obtained different numbers of malnutrition cases using the two methods, MUAC <11.5cm and WHZ ≤ -3 SD.

In previous studies, there were indeed differing results. One mentioned that MUAC was suitable for the diagnosis of severe acute malnutrition.[13] However, others stated that several cases of severe acute malnutrition were not diagnosed if MUAC was used singly as an anthropometric indicator for screening for acute malnutrition.[14]

In several studies there appeared to be discrepancies between malnourished children using WHZ or MUAC criteria. A previous study mentioned that MUAC and WHZ identified different groups of severe acute malnutrition. Also, the use of WHZ resulted in finding a higher prevalence of severe acute malnutrition compared to MUAC, as >90% of children with WHZ <-3 SD were not identified by MUAC <115 mm, while 80% of children with MUAC <115 mm were not detected by WHZ <-3 SD.[15] In addition, Fernandez et al. reported that among 34,937 children between the ages of 6 and 59 months obtained from 39 nutritional surveys, 75% of children with WHZ <-3 SD were not identified by MUAC <115 mm.[16]

When an individual loses weight, major losses of fat and muscle mass occur, so the MUAC and overall body mass would be affected. This raises the question as to why there is such a marked difference in diagnosing severe malnutrition by the two indicators, WHZ and MUAC.[17]

A possible explanation of this problem is that first, in contrast to WHZ, the diagnosis of acute malnutrition based on MUAC depends on one absolute cut-off point that is independent of age, height, and sex. As the child grows taller, weight and MUAC also increase, although to a different degree; children with the same WHZ tend to be below the absolute cut-off point of MUAC if they are shorter or younger. Thus, those who are diagnosed with malnutrition by MUAC tend to be younger than those who are diagnosed with malnutrition by WHZ.[13,14,18] Second, more children with shorter stature will not be diagnosed with severe malnutrition using MUAC, because the child is still in the stunting process. If nutritional management is done with an adequate intake of energy, then the rate of weight loss will stop/improve, but the rate of decrease in body length remains constant, so many children with normal MUAC are found and WHZ is improved. This is consistent with the WHZ, WAZ, and HAZ patterns presented in the previous study.[19] However, if inadequate nutrition affects longitudinal growth, then a positive relationship between nutritional status and stature should be found.[20] Third, racial differences impact the distribution of fat throughout the body in populations that normally live in the same environment. In groups where fat is more dominant in the limbs than the trunk, the value of MUAC increases relative to WHZ, whereas, if most of the fat is in the trunk, then the WHZ can be disproportionately increased. Although MUAC is a relatively good indicator of total body fat in children, MUAC is a poor indicator for describing fat-free tissue, especially muscle. Thus, muscle and fat loss can affect MUAC and WHZ differently.[21-23] Fourth, out of 853 under-fives with complete data in our study, short stature was noted in only 15.6%, far fewer compared to under-fives with normal stature (82.8%). The number of short (12.4%) and very short stature (3.2%) under-fives was also lower than the results of the *Indonesian Basic Health Research Report 2013* (*Riskesdas 2013*), which were 18.1% and 19.2%, respectively.[1] In addition, only 8.3% of under-fives had undernutrition and 2.8% had malnutrition in our study compared to the *Indonesian Basic Health Research Report 2013*, with percentages of thin under-fives of

6.8% and very thin under-fives of 5.3%.[1] There were also far fewer under-fives with undernutrition and malnutrition in our study (11.1%) than well-nourished under-fives (85.6%). We also noted that only 1.9% of our under-fives had weight/age ≤ -3 SD (*BGM*) in our study, compared to 5.7% in the *Indonesian Basic Health Research Report 2013*.[1] Thus, the under-fives who came to the posyandus mostly had normal weight, normal stature, and well-nourished status.[1]

The relationship between MUAC and WHZ is far more complicated than it appears. Although absolute MUAC values can identify malnutrition in younger and shorter children, further analysis must be done as to whether MUAC or WHZ can be used as criteria to assess increased risk of death and complications of malnutrition in older children.[13,24]

Previous study on the use of MUAC and WHZ in malnutrition screening was mostly carried out in countries with higher malnutrition rates and lower socioeconomic levels of society, such as some countries in the African continent that are categorized as poor. The high number of infections and the lack of guaranteed food availability are factors that cause high rates of malnutrition, both moderate and severe. A study mentioned that age had an important role in MUAC and WHZ differences in identifying children with malnutrition, which is related to food insecurity.[25] In younger children, the incidence of malnutrition is an indication of a disease accompanied by an inadequate intake process, whereas in older children malnutrition is more likely to occur due to certain periods of a lack of food availability in these countries. During periods of food shortages, older children tend to experience greater increases in acute malnutrition than younger children. The use of a single anthropometric indicator can mask the actual amount of malnutrition. Therefore, it is recommended to use both anthropometric indicators, MUAC and WHZ, to be able to correctly identify malnutrition for all age groups.[26]

Another study noted that the two indicators (MUAC and WHZ) complement each other in identifying children with increased risk of death from malnutrition. This finding was supported by the observation that children with deficits in both MUAC and WHZ have a worse prognosis than those who only have a single anthropometric deficit.[27]

Diagnostic testing is a technique for assessing the accuracy of new diagnostic modalities compared to standard diagnostic modalities, which are referred to as gold standards. New diagnostic tests must promise benefits, for example, less expensive, easier, faster, and less invasive compared to the gold standard, even though the sensitivity and specificity are (slightly) lower. The development of diagnostic tests can have several objectives, including to establish the diagnosis, as well as to fulfill the need for screening, treatment, and epidemiological studies.[28]

The results obtained from the comparison of standard and new diagnostic tests are sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios. The purpose of a diagnostic test should be determined. For screening purposes, diagnostic tests with high sensitivity are required. The sensitivity of a new diagnostic test shows the ability of the diagnostic tool to correctly detect a disease, while the specificity indicates the ability to determine that the subject does not have the condition.[29]

Previous studies have also assessed MUAC as a method of determining nutritional status in the community. These studies compared anthropometric measurements with the results of clinical evaluations or other methods besides anthropometry. In other studies, the sensitivity and specificity of MUAC compared to other anthropometric indicators were also evaluated.[16,30-32]

In our study, a diagnostic test for severe malnutrition screening was done to compare MUAC ≤11.5 cm to the gold standard WHZ ≤ -3 SD. The MUAC sensitivity obtained by the cadre was 12.5% and by researchers was 16.7, while the specificity of MUAC measurement by the cadre was 99.9% and by researchers was 99.6%, for MUAC cut off 11.5 cm. These results indicate that MUAC ≤ 11.5 cm cannot be used for screening severe malnutrition because it is not sensitive, even though its specificity is good. The MUAC sensitivity of 12.5% and 17% shows that a MUAC cut-off of 11.5cm can only detect 12.5-17% of children with severe malnutrition, while the other 83-87.5% who actually experience severe malnutrition are not detected, leading to a high number of false negatives despite the low number of false positives due to its high specificity of almost 100%. Such high MUAC specificity of the 11.5 cm cut-off indicates that children with MUAC> 11.5 cm do not really suffer

from severe malnutrition.

Our results are in agreement with a study that compared MUAC to WHZ in 5,751 children aged less than 5 years in Senegal. The sensitivity was 5.9% and specificity was 99%, when using a combination of diagnostic criteria for severe malnutrition WHZ <-3 SD and MUAC <115 mm. However, if they used only MUAC <115 mm as the diagnostic criteria, the sensitivity was 13.2% and specificity was 96.9%. In this study they also tested the sensitivity and specificity of various MUAC cut-off values. When using a MUAC cut-off of 112 mm, the sensitivity to diagnose acute malnutrition was 6% and specificity 99.1%. A MUAC cut-off of 119 mm, had 14.9% sensitivity and 96.9% specificity. Therefore, MUAC has better ability to diagnose severe acute malnutrition with a higher risk of complication than WHZ. In addition, there was no benefit to using a combination of WHZ and MUAC criteria to screen for children with severe acute malnutrition.[31]

Since the 11.5 cm MUAC cut-off showed a low sensitivity value, a ROC curve was used to determine the best MUAC cut-off value for screening, which was expected to have high sensitivity and specificity. The ROC curve showed an optimal MUAC cut-off of 14 cm with a 95.8% sensitivity, 80.3% specificity, 12.4% positive predictive value, and 99.9% negative predictive value. This is in accordance with a previous study in West Nigeria that assessed sensitivity, specificity, positive predictive value, and negative predictive value of MUAC in children aged 12-59 months, compared to WHZ as the gold standard. A 13.5 cm MUAC cut-off had 20% sensitivity and 95.3%specificity. Their ROC curve showed an optimal MUAC cut-off value of 15.5 cm, with 80% sensitivity and 53.5% specificity. Therefore, they recommended to increase the MUAC cut-off value for screening severe malnutrition patients under 5 years old.[32]

A limitation of our study was the small number of severe malnutrition cases of only 2.8%. Hence, our sample cannot reliably be used to determine MUAC cut-off point to screen for severe malnutrition. In addition, subjects from only one city were included, so they did not represent the actual number of cases of severe malnutrition in the country. Despite these limitations, MUAC can still be used as an indicator examination that is quite simple and easy for the cadres to use in order to find cases of malnutrition that need to be referred immediately to get appropriate treatment.

In conclusion, MUAC measurement with an 11.5 cm cut-off cannot be used for screening severe malnutrition cases in Posyandu because it lacks sensitivity, despite its good specificity. In this study, 14 cm is the best MUAC cut-off value for screening underfives with severe malnutrition.

Conflict of Interest

None declared.

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Case Report

Long-term follow up of a tuberous sclerosis patient: evaluation of anti-epileptic drugs and self- management support therapy

Anindya Diwasasri[1], Sunartini[1], Retno Sutomo[1], Retno Danarti[2]

Tuberous sclerosis (TSC) (OMIM 191100) is an inherited, autosomal dominant disorder affecting multiple organ systems.[1] A genetic mutation in one of the tumor suppressor gene (TSG) alleles causes tumor growth in various organ systems. Tuberous sclerosis can be found in people of all races, and does not differ in men and women, with an incidence 1 in 6,000 births and prevalence of 1 in 20,000.[1-3] Although the prevalence is quite high, diagnosing this disorder is often difficult and delayed due to diverse disease manifestations and varied age at onset.

Tuberous sclerosis (TSC) is associated with significant disease burden and has a considerable impact on quality of life.[4] Individuals with tuberous sclerosis need multidisciplinary treatment by experts because of the various clinic spectrums. To date, no single therapy can cure tuberous sclerosis. Management of tuberous sclerosis is done with the aims of helping patients achieve better quality of life, minimizing complications, and avoiding side effects of drugs.[3-6] With early detection, aggressive monitoring, and handling emerging symptoms, individuals with tuberous sclerosis may have longer life expectancy and learn independent survival skills.[3,6-8] Here we present an 18-month follow-up case report on a patient with tuberous sclerosis and intractable epilepsy, focusing on medical aspects and quality of life. **[Paediatr Indones. 2020;60:53-9 ; doi: http://dx.doi.org/10.14238/pi60.1.2020.53-60].**

Keywords: *tuberous sclerosis; anti-epileptic drug; self management support; quality of life*

The Case

A 13-year-old boy was diagnosed with epilepsy at the age of 8 years, and received regular valproic acid therapy from that time. Despite receiving anti-epileptic monotherapy, seizures continued to recur every 1-2 weeks and the frequency of seizures increased. At the age of 10 years, seizures occurred almost everyday, so carbamazepine was added to the valproic acid treatment. An electroencephalography

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Table 1. Clinical findings in the patient

Major criteria	The patient	Minor criteria	The patient
Hypomelanotic macules > 3, minimum diameter 5mm	Macules > 5, diameter 1-3cm	Confetti skin lesion	Multiple, 1-3mm
Angiofibroma or fibrous cephalic plaque	Facial angiofibroma (+)	Dental enamel pits >3	Not checked yet
Ungual fibroma > 2	Found	Intraoral fibroma	Not found
Shagreen patch	Multiple, in back and knees	Retinal achromic patch	Not found
Multiple retinal hamartomas	Not found	Multiple renal cyst	Not found
Cortical dysplasia	(+)	Non renal hamartoma	Not found
Subependymal nodules	Not found		
Subependymal giant cell astrocytoma	Not found		
Cardiac rhabdomyoma	Not checked yet		
Lymphangioleiomyomatosis of the lung	Not checked yet		
Renal angiomyolipoma	Not found		

*Definitive diagnoses: fulfilling 2 major criteria OR 1 major with >2 minor criteria. Possible diagnosis: fulfilling 1 major criteria OR >2 minor criteria.

(EEG) examination performed when he was 8 years of age in September 2014, revealed an epileptiform wave in all leads, and an MRI scan of the head revealed the presence of calcification in the temporal lobes of the left caudatus and ventriculomegaly with cavum septum pelusidum. Head MRI revealed some tubers in brain parenchyme and sub-ependymal nodule at the wall of the lateral ventricle (**Figure 1**).

The child had a facial skin lesion recognized by his parents at the age of 9 years, one year after a seizure (**Figure 2**). There were some white patches on the the body and back (**Figure 3a**). The initial tracking of involvement of other organ systems was done when he was 10-year-old, with no evidence of retinal hamartoma, dental pit, gingival fibroma, or abnormalities in the kidneys and intraabdominal organs in abdominal CT scan.

No family members had similar disease. The child had delayed gross motor and speech skills (walking at the age of 17 months and speaking fluently at the age of 3 years). He dropped out of school in grade 5 due to daily seizures. In mid-2014, the patient was diagnosed with tuberous sclerosis (fulfilled ≥ 2 major diagnostic criteria according to the 2012 consensus) and intractable epilepsy. Anti-epileptic drugs given were carbamazepine 5mg/kg body weight (BW)/12 hours, clonazepam 0.05mg/kg BW/12 hours, and valproic acid 20mg/kg BW/day.

Observations were conducted prospectively for 18 months (July 2016-January 2018). The dependent variables observed were epilepsy, clinical manifestations in organ systems, and patient quality of life (see **Appendix**). Independent variables observed were anti-epileptic drug doses and self-management support for the patient.

The patient had clinical manifestations of tuberous sclerosis complex (TSC) in the form of distinctive skin lesions including ash leaf macules, facial angiofibroma, and shagreen patch that was recognized at the age of 9 years. During our 18-month

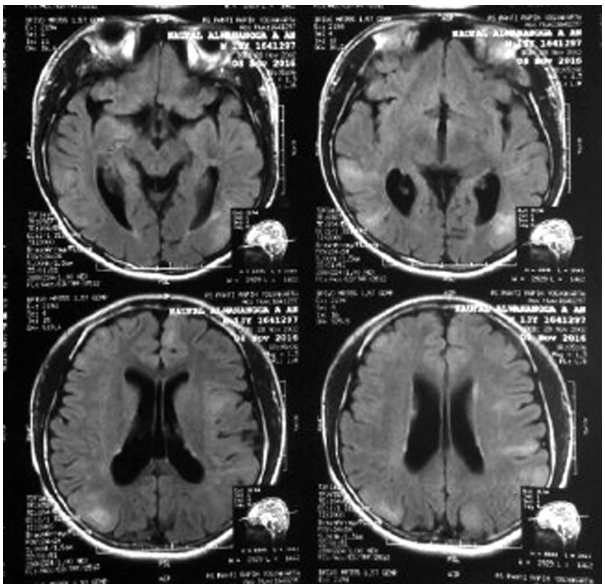

Figure 1. MRI showed some tubers in brain parenchyme (white circles) and sub-ependymal nodule at the wall of the lateral ventricle (red circle)

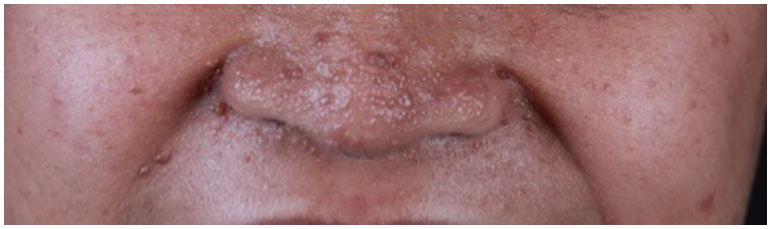

Figure 2. Skin lesions found in our patient: angiofibroma and fibrous cephalic plaque

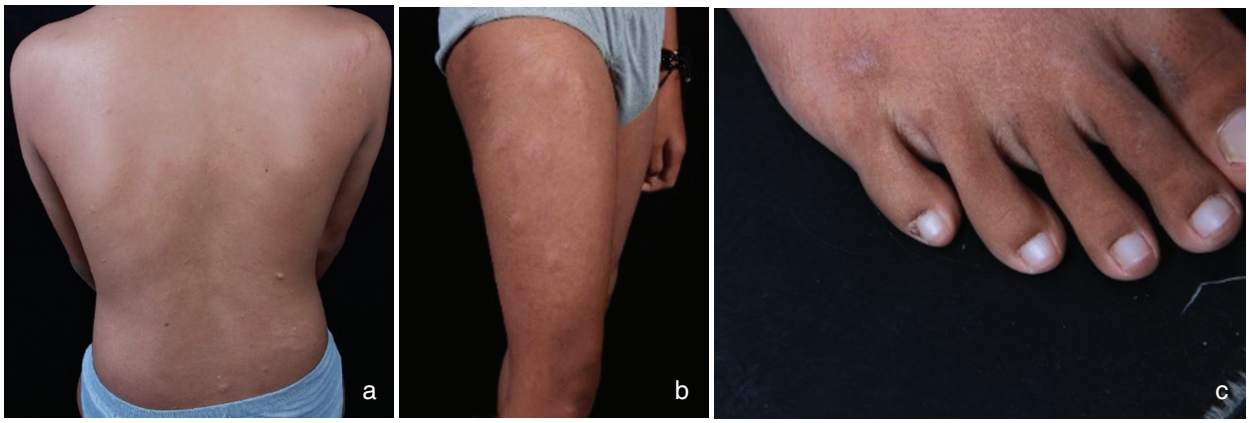

Figure 3. Multiple shagreen patches and ashleaf macules on the (a) back and (b) leg; (c) ungual fibroma on nails

observation, the shagreen patch enlarged and the facial angiofibroma increased in number (**Figure 2** and **Figure 3**).

Seizures began at age 8, with focal and secondary generalized types. During the observation, seizures were still present even with three anti-epileptic drugs (carbamazepine, clonazepam, and valproic acid), thus the patient was considered to have intractable epilepsy. At the beginning of the observation, our patient experienced seizures almost everyday. Topiramate was added to his daily regimen at an adjusted dose. During the observation, our patient showed improvement in clinical symptoms with an increased topiramate dose of 3mg/kg BW/day. The seizures resolved with valproic acid 60mg/kg BW/day, carbamazepine 18 mg/kg BW/day, and topiramate 3 mg/kg BW/day (**Figure 4** and **Figure 5**). The last EEG monitoring in October 16, 2017 showed an improvement from the previous one, although diffuse epileptiform waves were still found (**Figure 6**).

Discussion

Epilepsy is found in 85% of TSC patients. Most epilepsy in TSC is refractory to anti-epileptic drugs.[9] Multi-organ abnormalities in TSC occur at specific ages. We observed the patient from 13 to 15 years of age. At this age, abnormalities that can arise include gum fibroma and dental pits, ungual fibroma, and the development of pre-existing subependymal nodule (SEN) to subependymal giant cell astrocytoma (SEGA). As stated in a previous study, TSC skin lesions can appear as early as the neonatal stage, yet they are difficult to detect even with Wood's lamp examination. A hypomelanotic macula is clearly visible at age of 5 years, while angiofibroma may be found starting at the age of 5-10 years.[9]

Renal manifestations such as polycystic kidney disease and renal angiomyolipoma can be found in 85% of adult TSC patients. Pulmonary lymphangioleiomyomatosis is present in 40-80% of adult female TSC patients, but in a smaller percentage of men. Cardiac abnormalities could appear from as early as 20 weeks of gestation, persist in neonatal period (80%),

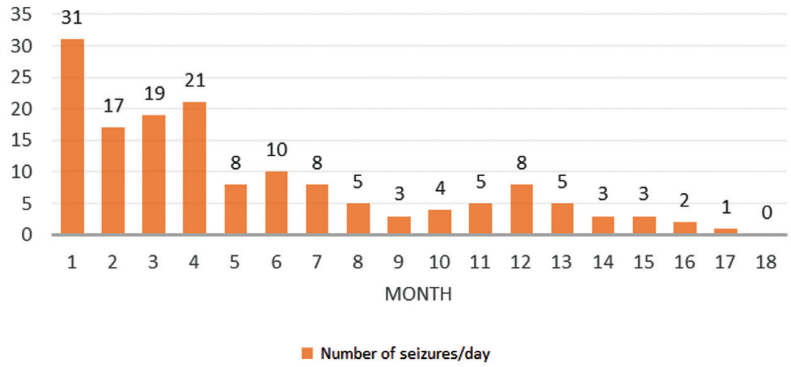

Figure 4. Frequency of seizures/month during the 18-month study

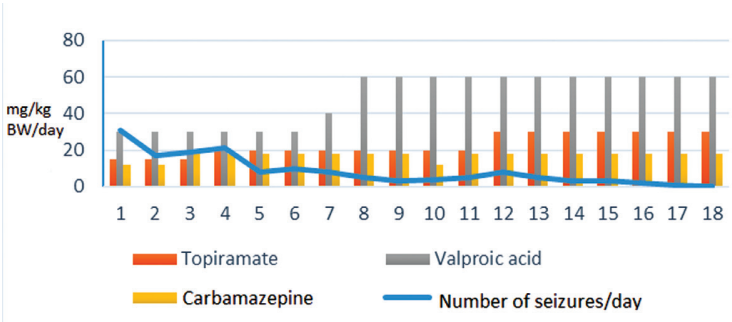

Figure 5. Decreasing frequency of seizures related to increasing doses of anti-epileptic drugs during the 18-month observation. Seizures resolved with valproic acid 60mg/kg BW/day, carbamazepine 18 mg/kg BW/day and topiramate 3 mg/kg BW/day

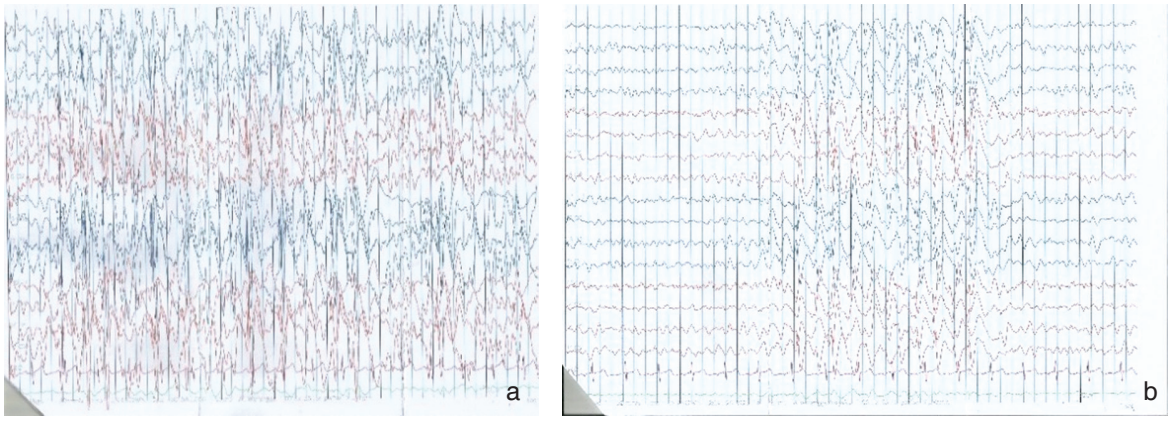

Figure 6. (a) Earlier EEG showed diffuse abnormal (hypsarrhythmia-like) epileptiform waves. (b) The EEG at the end of the study showed improvement of the epileptiform waves

then decrease over time.[10] In adolescence, heart abnormalities may be found, especially disturbances in heart rhythm. We found no dental pit or gum fibroma, over a series of physical examinations. However, when our patient was 14 years of age, we found periungual fibromas. There were multiple tubers in the cortex and subependymal nodule (SEN) in the last MRI examination. Chest x-ray, electrocardiography, and abdominal CT examination revealed no abnormalities in the lungs, heart rhythm, or kidneys.

Topiramate is a new drug effective for treating focal seizures with or without secondary generalization in tuberous sclerosis patients.[2,3] Topiramate's mechanisms of action are to inhibit voltage-dependent sodium channels, increase GABA activity in GABA(A) receptors, and act as a NMDA-glutamate receptor antagonist. Topiramate is currently used as an add-on therapy in focal and secondary generalized epilepsy at a dose of 2.5 - 4.5 mg/kg BW/day, divided into two doses, with a maximum dose of 200mg/12 hours.[4]

Self-management support should be provided by the government through a community health system for individuals and families dealing with chronic diseases to improve health outcomes and reduce utilization, cost, and caregiver burden.[11] The system involves collaboration of patients, families, and health care providers.[12] In adult populations, self-management support was shown to improve health outcomes and patient independence.[13,14] Self-management support is one of the pillars in the primary care services provided to patients with chronic disease.[13] Although considered to be a best practice in the care of adults with chronic conditions, comprehensive self-management support (SMS) programs are not typically available in pediatric practices. Self management support has not been widely developed for children and adolescents and has not been formally integrated in our health services.

Our patient was seizure-free at the end of our study. As his physical, emotional, and social functions improved, the child returned to school. The quality of life of tuberous sclerosis patients is significantly reduced compared to the normal population, with the psychosocial aspect most affected.[15] Neuropsychological intervention is needed to improve patient quality of life,[16] as the quality of life in such patients is mainly influenced by neurological and neuropsychiatric manifestations. Epilepsy is the main factor affecting the patient quality of life. If the epilepsy can be controlled, it can improve the patient's quality of life. As the main outcome of this study, we used PedsQL inventory to evaluate the quality of life of our patient. After intervention, his Peds QL score showed improvement. As such, with good medication compliance and appropriate anti-epileptic drugs doses, we could control the epilepsy symptoms so that he achieved a better quality of life at the end of the observation.[4,7,8,17,18]

Conflict of interest

None declared.

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Appendix: Summaries of evaluation and monitoring during study

No.	Variables	Basic data	Intervention	Results
1	Manifestations of the nervous system	Head CT scan: calcification in temporal lobes and caudate nucleus sinistra, ventriculomegaly with cavum septum pellucidum	1. Physical examination 2. Head MRI every 1-2 years	Head MRI results: No visible nodular hypointense lesion on T1W1, hyperintense on T2W1 nor hypointense on FLAIR Impression: no / not yet seen tuberous sclerosis cyst. Cavum septum pellucidum
2	Skin manifestations	Multiple facial angiofibroma Multiple shagreen patches on the right thigh and waist Ashleaf macule on right leg	Observation and monitoring	The amount and the size of facial angiofibromas increased Shagreen patch size enlarged Found ungual fibroma on the right and left foot toenails Laser treatment for facial angiofibroma was planned to patient
3	Cardiac manifestations	No complaints of chest pain. On physical examination, there was no interruption of heart rhythm and heart sound was within normal limits. ECG and echocardiography have not yet been done	1. Physical examination 2. ECG 3. Echocardiography	No impaired arrhythmia, heart sound within normal limits. ECG: normal sinus rhythm; no conduction disorder or arrhythmia.
4	Pulmonary manifestations	No complaints of shortness of breath. Thoracic X-ray and CT scan had not yet been done	1. Physical examination 2. Thoracic X-ray examination	No complaints of breathlessness and thoracic X-ray examination was normal

Appendix: Summaries of evaluation and monitoring during study (continued)

No.	Variables	Basic data	Intervention	Results
5	Self-management support for chronic disease	1. Patients and families did not understand the disease 2. Parents did not have daily diaries of drugs taken by children 3. The parents objected to follow-up visits to the Pediatric Polyclinic, RSUP Dr. Sardjito, due to the tiered referral process and the long queue 4. There was no daily record of the child's major complaints or other complaints 5. The child had not attended school since the age of 10 because he was embarrassed by his seizures 6. The child tended to be alone at home, found it difficult to socialize, and had no peers	1. Overarching care processes 2. Informational support 3. Peer support 4. Coaching support 5. Informational and technological support 6. Family daily management support	1. Patients and families were able to understand the illnesses with regards to etiology, disease process, complications and prognosis 2. The child had a diary to record prescribed medications and medication schedule 3. Parents routinely brought the patient to the neurology clinic every month and to the child psychologist every 3 months 4. The child had a diary to record the frequency and type of seizures 5. The child had returned to school with the goal of achieving national equivalency education (package A) 6. The child was more extroverted and able to communicate with peers and people outside the family. He felt more confident. From the last examination by a psychologist, the child no longer experienced anxiety disorders or depression 7. Parents and children communicated with the doctor at any time if needed and can easily accessed information related to the child's health
6	Quality of life	1. Child General QoL: 65.21 2. Mother General QoL: 56.45 Impression: poor quality of life	1. Pharmacological therapy with anti-epilepsy drugs 2. Self-management support 3. Behavioral therapy 4. PedsQL score re-evaluation	1. Child General QoL: 71.4 2. Mother General QoL: 63.75 Impression: the quality of life according to the perception of the child and mother was quite good, and better than at the initial examination

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Original Article

Predictors of prolonged stay in the pediatric intensive care unit

Yudha Fadhol Arafah, Indah K. Murni, Desy Rusmawatiningtyas

Abstract

Background Prolonged stay in the pediatric intensive care unit (PICU) reflects not only disease severity and patient health status, but also the performance and quality of patient care.

Objective To to determine whether surgical procedure, severe malnourishment, cardiovascular condition, sepsis, and ventilator use were the predictors of prolonged PICU stay.

Methods This nested, case-control study was conducted with secondary data from medical records of pediatric inpatients at Dr. Sardjito General Hospital, Yogyakarta, Indonesia. We included pediatric patients aged 1 month-18 years treated in the PICU between January 1 to December 31, 2018. Predictors of prolonged stay were identified including surgical procedures, severe malnourishment, cardiovascular conditions, sepsis, and ventilator use. Logistic regression was used to identify independent predictors.

Results Subjects' overall median age was 3.12 (IQR 0.76-18.8) years and the male to female ratio was 1:1. Median duration of ventilator use was 4 (IQR 1-21) days. The most common diagnosis was neurological disease (26.7%). Multivariate analysis showed that surgical procedure (OR 5.75; 95%CI 2.06 to 14.61) was statistically significant as an independent predictor of prolonged PICU stay.

Conclusion Surgical procedure is the significant predictor of prolonged stay in PICU. **[Paediatr Indones. 2020;60:37-41; doi: http://dx.doi.org/10.14238/pi60.1.2020.37-41]**.

Keywords: *prolonged stay; predictor; PICU*

The pediatric intensive care unit (PICU) is designed to treat children with life-threatening conditions, trauma, recovery from surgery, or who need intensive treatment, comprehensive observation, or special care.[1] The reported mortality rate of PICU patients in the United States was 20%, whereas the global mortality rate was 25% per year. Mortality may be caused by infection, prolonged stay, and/or inadequate treatment.[2]

Prolonged PICU stay is reflective of disease severity and patient health status. It also indirectly describes the performance and quality of the PICU.[3] Prolonged PICU stay has been well studied in developed countries, but few studies have been conducted in low-to-middle income countries like Indonesia. Previous studies have also lacked agreement on predictors of prolonged PICU stay. A study noted that heart abnormalities were not predictors of prolonged stay,[4] but another study showed the opposite.[5] In addition, another previous study concluded that sepsis was

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not a predictor of prolonged stay,[5] but this result was not in agreement with results from other previous studies.[6,7]

We aimed to determine whether surgical procedure, severe malnourishment, cardiovascular condition, sepsis, and ventilator use were predictors of prolonged PICU stay in children at Dr Sardjito General Hospital, Yogyakarta.

Methods

A nested, case-control study was done using secondary data. Inclusion criteria were PICU patients aged 1 month to 18 years who were treated from January 1 until December 31, 2018, in Dr. Sardjito General Hospital, Yogyakarta. The exclusion criteria were children with incomplete medical records or who were discharged against medical advice. The outcome was prolonged stay. Prolonged stay was defined as length of stay (LoS) in the PICU ≥7 days starting from PICU admission until discharge, transfer to the ward, or death. The independent variables were surgical procedures, severe malnourishment, cardiovascular conditions, sepsis, and ventilator use. A surgical procedure was defined when patient had undergone major non-cardiac surgical procedure. Nutritional status was determined based on the *World Health Organization* (WHO) z-score growth charts. Subjects were classified as severely malnourished if weight-for-height or body mass index (BMI)-for-age was <-3SD. Sepsis was determined based on warning signs, clinical manifestatitions, and evidence of organ failure by PELOD-2 score.[8,9] Cardiovascular condition was defined as having congenital heart disease (acyanotic or cyanotic) that was confirmed by echocardiography. We used 0.05 confidence level and 80% power to calculate for the minimum required sample size.

Data were analyzed using *Software Package for Social Science (SPSS) version 11.0 for Mac OS Sierra*. Chi-square test was used for bivariate analysis. Variables with P values <0.05 were further analyzed by multivariate using logistic regression method to find potential correlations with prolonged PICU stay. The risk of prolonged stay was calculated using odds ratios and confidence intervals. This study received approval from the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Gadjah Mada University/ Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

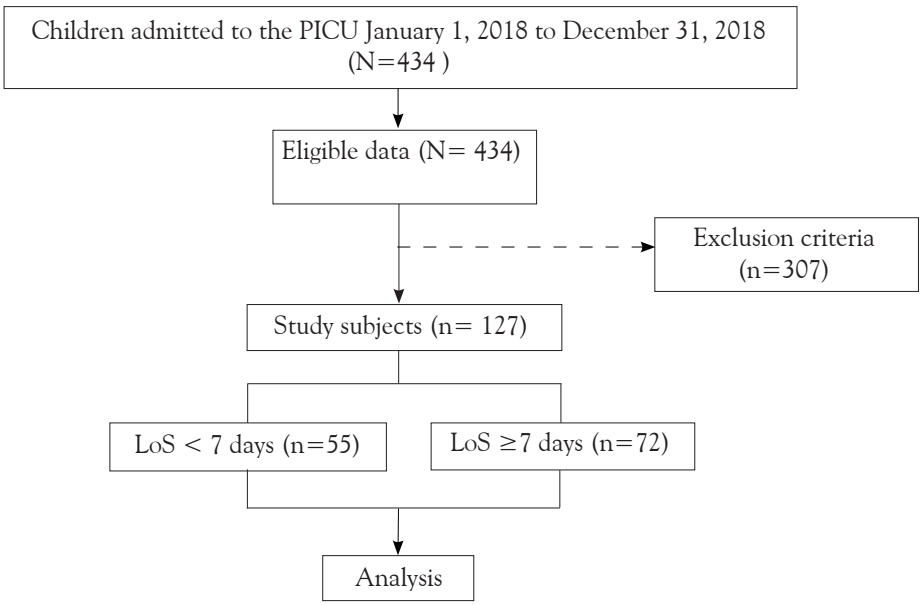

Figure 1. Study flow diagram

Results

Four hundred thirty-four patients fulfilled the inclusion criteria, but 127 were analyzed (**Figure 1**). The characteristics of subjects are shown in **Table 1**.

The bivariate analysis results are presented in **Table 2**. Surgical procedures (OR 6.17; 95%CI 2.34 to 16.25; P<0.001) and sepsis (OR 0.23; 95%CI 0.07 to 0.78; P=0.01) were significant predictors of prolonged PICU stay. Further multivariate analysis results are presented in **Table 3**. Surgical procedures

Table 1. Characteristics of subjects

Characteristics	Admitted to PICU		
	Total (N=127)	LoS<7 days (n=55)	LoS ≥7 days (n=72)
Median age (range), years	3.12 (0.76-18.8)		
Sex, n (%)			
Male	62 (48.8)	33 (60)	29 (40.3)
Female	65 (51.2)	22 (40)	43 (59.7)
Severe malnourishment, n (%)	21(16.5)	6 (10.9)	15 (20.8)
Diagnosis, n (%)			
Neurology	34 (26.7)	17 (30.9)	17 (23.6)
Respiratory	28 (22.0)	13 (23.6)	15 (20.8)
Surgery	24 (18.9)	7 (12.7)	17 (23.6)
Gastro-hepatology	9 (7.0)	2 (3.6)	7 (9.7)
Infection	9 (7.0)	7 (12.7)	2 (2.8)
Hematology-oncology	8 (6.3)	5 (9.1)	3 (4.2)
Nephrology	7 (5.5)	2 (3.6)	5 (6.9)
Cardiology	4 (3.1)	0 (0.0)	4 (5.6)
Immunology	2 (1.5)	0 (0.0)	2 (2.8)
Endocrinology	2 (1.5)	2 (3.6)	0 (0.0)
Surgical procedures, n (%)	n=37	n=6	n=31
Laparotomy	21 (56.7)	3 (5.5)	18 (25.0)
Craniotomy	6 (16.2)	2 (3.6)	4 (5.6)
Thoracotomy	6 (16.2)	0 (0.0)	6 (8.3)
Tracheostomy	2 (0.05)	0 (0.0)	2 (2.8)
Trans-anal surgery	1 (0.03)	1 (1.8)	0 (0.0)
Laparoscopy	1 (0.03)	0 (0.0)	1 (1.4)
Median duration of ventilator use (range), days	4 (1-21)		

Table 2. Bivariate analysis of possible predictors of prolonged PICU stay

Variables	LoS <7 days (n=55)	LoS ≥7 days (n=72)	OR	95% CI	P value
Surgical procedures					
No	49 (89)	41 (57)	6.17	2.34 to 16.25	<0.001
Yes	6 (11)	31 (43)			
Severe malnourishment					
No	49 (89)	57 (79)	2.14	0.77 to 5.96	0.14
Yes	6 (11)	15 (21)			
Cardiovascular condition					
No	48 (87)	59 (82)	1.51	0.55 to 4.08	0.41
Yes	7 (23)	13 (18)			
Sepsis					
No	44 (80)	68 (94)	0.23	0.07 to 0.78	0.01
Yes	11 (20)	4 (6)			
Ventilator					
No	9 (16)	18 (25)	0.58	0.24 to 1.43	0.24
Yes	46 (84)	54 (75)			

Table 3. Multivariate analysis of possible predictors of prolonged PICU stay

Variables	OR	95%CI	P value
Surgical procedure	5.75	2.06 to 14.61	<0.001
Sepsis	0.32	0.09 to 1.12	0.07

(OR 5.75; 95%CI 2.06 to 14.61; P<0.001) remained a statistically significant predictor of prolonged stay. However, severe malnourishment, cardiovascular condition, sepsis, and ventilator use were not significant predictors of prolonged stay.

Discussion

Surgical procedures significantly increased the risk of prolonged PICU stay by almost six times. The outcome of this study is the first to present non-cardiac surgical procedures as a predictor of prolonged stay in the pediatric population. In a study of adults who underwent non-cardiac surgical procedures in Portugal, surgical procedures may have affected the LoS of patients in the ICU due to other co-factors, such as the choice of sedation, amount of intravenous fluids given during surgery, and the severity of the surgical procedure itself.[8,10]

In our study, although severe malnourishment was not a statistically significant predictor of prolonged PICU stay, this associated with a 2-fold increase in the probability of prolonged PICU stay provided that the sample size is sufficiently large. This is similar to a previous study reported that severe malnourishment was significantly related to PICU LoS.[11] Furthermore, children with severe malnourishment who received PICU treatment had 6.8 times higher risk of mortality compared to those with normal nutritional status.[12]

In our study, cardiovascular condition was not a significant predictor of prolonged PICU stay. This finding was consistent a previous study that also noted that cardiovascular condition was unrelated to prolonged PICU stay (P=0.08).[4] This finding of our study is unrelated due to a distribution imbalance which manifest to the outcomes. If the outcomes are evenly distributed and the sample size is large, the result might be statistically significant.

In our study, sepsis was also not a significant predictor of prolonged PICU stay, consistent with a previous study.[5] Children admitted to PICU with sepsis in our study tended to have shorter length of stay. This might because among those who had sepsis might develop septic shock and the mortality was high.[9] In a previous study conducted in the same PICU revealed that the median length of stay of children with septic shock was about 4 days.[9]

We also did not observe that a ventilator usage was not a significant predictor of prolonged PICU stay. The median ventilator use among patients in our PICU was 4 days. This finding had general agreement with other studies who both reported that the length of ventilator use for ≥7 days was associated with longer PICU stay.[10,13]

The main limitation of this study was the use of secondary data collected from medical records and the predictors simply be selected from the available data. This study was conducted in a single national referral hospital that may limit the generalizability of the study. Further cohort studies with evenly distributed samples are needed.

In conclusion, surgical procedure is a predictor of prolonged PICU LoS. Therefore, management of children who undergo surgical procedures should be improved so that PICU LoS can be reduced.

Conflict of Interest

None declared.

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Original Article

Multilevel survival analysis for under-fives in Indonesia 2015

Linta Ifada[1], Mieke Nurmalasari[2], Setia Pramana[1,3]

Abstract

Background Marking the end of the *Millennium Development Goals* (MDGs) era, governments continue their plans via the *Sustainable Development Goals* (SDGs). One of the MDGs that has continued is the reduction in under-five mortality. Even though the trend of under-five mortality in Indonesia is decreasing, more efforts are needed to reduce the under-five mortality rate.

Objective To determine the individual and contextual factors of the under-five survival rate and to assess for possible characteristics that may lead to variance among regencies in Indonesia.

Methods Data from *2015 Intercensal Population Survey (Survei Penduduk Antar Sensus/SUPAS 2015)* in Indonesia were analyzed using multilevel survival analysis. The *Intercensal Population Survey* covers all regions in Indonesia up to the regency level. Data were collected by direct interviews of selected household members, with regards to demographic and household characteristics, including births and deaths of under-fives. Our sample population was limited to all under-fives who were born and died during the 2010-2015 period. The number of subjects analyzed was 219,413 after exclusion of children with incomplete data.

Results Individual factors associated with under-five survival rate were maternal education, maternal age at first birth, work status, sex, previous birth interval, type of birth, place of residence, and sanitation level. The contextual factor (health care facility ratio per 1000 under-fives per regency) was not associated with under-five survival rate. The 5.27% variance can be explained by the differing characteristics among regencies.

Conclusion The individual factors affecting the survival of under-fives are maternal education, maternal age at first birth, maternal work status, sex, previous birth interval, type of birth, place of residence, and sanitation level. **[Paediatr Indones. 2020;60:103-10; doi: http://dx.doi.org/10.14238/pi60.2.2020.103-10]**.

Keywords: *survival; under-five; individual factor; contextual factor*

Welfare, health, and well-being are rights of every child in the world. The fate of the future is in the hands of these children. To achieve a brighter future, we need to prepare our children to be healthy and educated in order to have a high-quality generation. Decreasing child mortality is one step to achieve that goal.

Child mortality is an important indicator for child health and welfare. In 2000, world leaders agreed on the *Millennium Development Goals* (MDGs). One of the goals was to urge the world to decrease under-five mortality by two-thirds in 2015. Great progress was made during those 15 years of effort. The under-five mortality has decreased and the chances of surviving the five first years of life keeps increasing. The worldwide under-five mortality rate has decreased as much as 53%, from 91 deaths in 1990 to 43 deaths in 2015 per 1000 livebirths. In the same period of time, under-five mortality has decreased from 12.7 million

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to 5.9 million each year.[1] One-third of the world (62 countries) has succeeded in decreasing the under-five mortality rate and met goal number 4 of the MDGs, including Indonesia. According to the *2015 UNICEF Report*, the under-five mortality rate in Indonesia had dropped to 27 deaths per 1000 livebirths, while the MDGs target for Indonesia was 28 deaths per 1000 livebirths. Although Indonesia looked like it had reached the goal, the report was based on estimated numbers.

Despite great progress, the MDGs target number 4 has still not been reached globally. The decline of the under-five mortality rate amounted to 53%, which was far from the target of decreasing two-thirds of the under-five mortality rate. If this trend continues, the world will only reach the target in 2026, which is a delay of more than a decade.[1]

As 2015 came to an end, world leaders wrapped up the MDGs program and focused on a new program, the *Sustainable Development Goals* (SDGs). SDGs are the continuation of the MDGs program in which the health of under-fives remains a concern. This aim is listed on the SDGs target number 3.2, which aims to decrease infant and under-five mortality to a maximum of 12 deaths per 1000 livebirths for neonatal mortality and 25 deaths per 1000 livebirths for under-fives at the end of 2030. [2]

In this study, we evaluated child health with a focus on under-five survival. The basic concept of social research is that individuals always interact with the group to which they belong. Both individuals and the group influence each other.[3] In addition to the group where individuals belong, under-five survival is also influenced by place of residence. The availability of healthcare facilities is an important factor in under-five survival. Therefore, determining under-five survival based only on individual factors may not be enough. There may be a community or contextual factor that affects under-five survival. Hence, we aimed to analyze under-five survival with regards to both individual and contextual factors using multilevel survival analysis.

Methods

We used secondary data from the *2015 Intercensal Population Survey* in Indonesia which was conducted by the *Central Bureau of Statistics*, Indonesia. *The Intercensal Population Survey* covers all regions in Indonesia up to the regency level. Data were collected by direct interviews of selected household members, with regards to demographic and household characteristics, including births and deaths of under-fives.

Our sample population was limited to all under-fives who were born and died during the 2010-2015 period, assuming that all demographic and household characteristics of the households stayed the same. We chose to focus on data for only a five-year interval in order to limit the likelihood of significant changes in demographic and household characteristics if we studied data from longer time intervals. The number of subjects analyzed was 219,413 after exclusion of children with incomplete data.

The number of healthcare facilities from each regency was secondary data obtained from the *Indonesian Ministry of Health*. We converted the data into ratios of the number of healthcare facilities per 1000 under-fives per regency. Unit of analysis in this study was the under-fives born between 2010-2015. The outcome variable was the risk of death for under-fives, as measured by under-five survival time from birth until death. In this study, the survival time is presented in months, ranging from 0 to 60 months.

The variables were categorized as individual and contextual factors. Individual factors consisted of maternal education, maternal age first birth, maternal employment status, sex of the child, previous birth interval, birth type, place of residence, source of drinking water, and sanitation level (private toilet, public toilet, or no toilet). The contextual factor consisted of only the ratio of healthcare facilities per 1000 under-fives per regency.

Maternal education status was defined as the last education which the mother had pursued. It was categorized into 3 categories: low, middle, and high. Mothers who only pursued at maximum of elementary schools fell into category 'low', including those who never attended school at all. Mothers who pursued until junior or high schools were categorized into category 'middle' and those who attended universities were categorized into category 'high'. Maternal age at first birth was also one of the individual factors. It was defined as the mother's age when gave birth to the first child. In this study, we categorized maternal age

at first birth into two different categories. The first category was for those who gave birth to the first child at age '20-35 years old', and the second category was for those who gave birth to the first child at '<20 years old or >35 years old'. Another maternal information that was used in this study was maternal employment status. The maternal employment status was obtained through mother's last week activities approach. In the *2015 Intercensal Population Survey* questionnaire, last week activities had four selections which were working, doing house chores, attending to school, and others. From these four selections, we categorized mothers who were working for the past week as 'employed', and the rest as 'unemployed', including mothers who were attending school at the time.

Variable sex referred to the sex of the child. It was categorized into 'male' and 'female'. Previous birth interval was the interval between the time when the child in this study was born and the child born exactly right before of the same mother. Time interval was calculated into months and categorized into 2 categories, which were '<24 months' and '≥24 months old'. If the child in this study was a first born child, it fell into '<24 months' category automatically. Another child information that went into account was whether the child was the result of 'single' birth or 'multiple' birth. While single birth was clearly defined as one-baby at one birth, multiple birth could be the result of twin, triplet, or quadruplet births.

In addition to those key variables, we also added three household variables. The first one was the place of residence. The importance of the place of residence was the availability of infrastructure and health care to support the delivery and child care. 'Urban' area apparently had better infrastructure and health care, while 'rural' area had less basic infrastructure and fewer health care. The second household variable was source of drinking water. It was divided into 2 categories, 'clean' and 'poor' source of drinking water. The availability of clean water was really important for the health. Poor source of drinking water was more likely to be contaminated with bacteria. Household which consuming water from bottled water, pipe water, protected well, or protected water spring and had >10 meters distance from landfill site was categorized as having clean source of drinking water. Meanwhile household which consuming from protected well or protected water spring but had ≤10 meters distance from landfill site would be categorized as having poor source of water. Poor source of water also included river water and rain water. The last household variable was sanitation. Variable sanitation in this study referred to the ownership of the toilet. In the *2015 Intercensal Population Survey* in Indonesia, selected household was asked whether they had a toilet or not. Households which had toilet and used only by household members were categorized as having 'private toilet'. Households that shared toilet with other households, or had to go to public toilet, were categorized as having 'public toilet'. Meanwhile, households that had no toilet facilities at all were categorized as having 'no toilet'.

We used both descriptive and inferential analyses for this study. Descriptive analyses are presented in tables and graphics to give the general picture about the characteristics of under-fives in Indonesia, shown in percentages in order to see into which categories the majority of under-fives fall. For the inferential analysis, we used a multilevel survival analysis which had two levels, individual and regency. We aimed to assess the effect of individual and contextual factors on the survival of under-fives, as well as to evaluate variations in under-five survival time caused by different characteristics among regencies.

The first step of this study was to choose the best model for the data using the akaike information criterion (AIC) produced by null models of exponential, gamma, lognormal, loglogistic, and Weibull distribution.[3,4] The null model produced the smallest AIC, so it was chosen as the best model, followed by assumption test for the chosen distribution and multilevel survival model estimation. The last step was to calculate the intraclass correlation (ICC) to assess for variations among regencies.

The purpose of this multilevel survival analysis was to assess for effects of individual and contextual factors on under-five survival. The smallest AIC was produced by a null model of Weibull distribution. The proportional hazard for assumption test showed that all explanatory variables met the criteria and could be included in the model. Random effect significance test was done to compare the usefulness of multilevel survival model to the usual survival model. The likelihood ratio was 1649.29 with P value < 0.0000. As such, we could reject the null hypothesis and conclude that there was a significant random effect to under-five survival in Indonesia. Also, multilevel

survival analysis was a better model for the data than the usual survival analysis. Subsequently, test was done to see if there was at least one explanatory variable with a significant effect on under-five survival. The value was calculated as shown below:

G^2 value of 2522.8 was larger than $X^2_{0.05,10}$=

$$G^2 = -2\ln\left[\frac{\text{Likelihood of null model}}{\text{Likelihood of conditional model}}\right]$$

$$= -2\ [-81949.288-(-80678.888)]$$
$$= 2522.8$$

18.307, so it could be used to reject the null hypothesis. With 5% significance level, we can conclude that there is at least one significant explanatory variable to under-five survival in Indonesia.

Variation among regencies was analyzed by intraclass correlation (ICC). According to the variation from the Weibull null model from Appendix 2, ICC was calculated as follows:

This ICC of 5.27% under-five survival variation

$$\rho = \frac{(\sigma^2_{\mu 0})}{(\sigma^2_{\mu 0} + \sigma^2_e)}$$
$$= \frac{0.1829546}{0.1829546 + 3.29} = 0.0527$$

was due to regencies' variations.

Results

During 2010-2015, 6.3% of under-fives in Indonesia died before reaching age five. Most of these died in their first year of life (89.8%), with the trend decreasing as the age increased. Overall, the average age at death for these under-fives was 0.26 years (3-4 months) (**Figure 1**). The percentages of under-fives who had died based on explanatory variables are shown in **Table 1**. The three highest mortality percentages were seen in those who were of multiple birth type, had low previous birth interval (<24 months), and no toilet.

Kaplan-Meier test revealed that the survival curve for under-fives with high maternal education was above the survival curves for middle and low

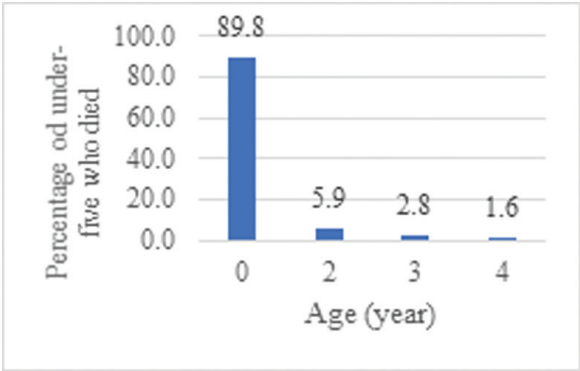

Figure 1. Percentage of under-fives in Indonesia who died from 2010-2015 by age at the time of death[5]

Table 1. Percentages of under-fives who died in 2010-2015 based on explanatory variables

Variables	Category	Percentage (%)
Maternal education status	Low	8.5
	Middle	5.4
	High	3.7
Maternal age at first birth	20-35 years	5.9
	<20 or >35 years	7.2
Maternal employment status	Unemployed	5.9
	Employed	6.8
Gender	Male	7.0
	Female	5.5
Previous birth interval	<24 months	14.3
	≥24 months	5.9
Birth type	Single	6.0
	Multiple	22.9
Place of residence	Rural	7.2
	Urban	4.9
Source of drinking water	Poor	6.1
	Adequate	6.5
Sanitation	Private toilet	5.5
	Public toilet	7.8
	No toilet	9.1

maternal education. Hence, under-fives whose mothers had high education were more likely to live longer than under-fives whose mothers had middle and low education (**Figure 2**). **Figure 3** shows that the survival curve for under-fives whose mothers' age at first birth was 20-35 years was above the survival curve for under-fives with whose mothers' age at first birth was <20 or >35 years. Hence, under-fives who had mothers who had an ideal age at first birth (20-35 years) were more likely to live longer than

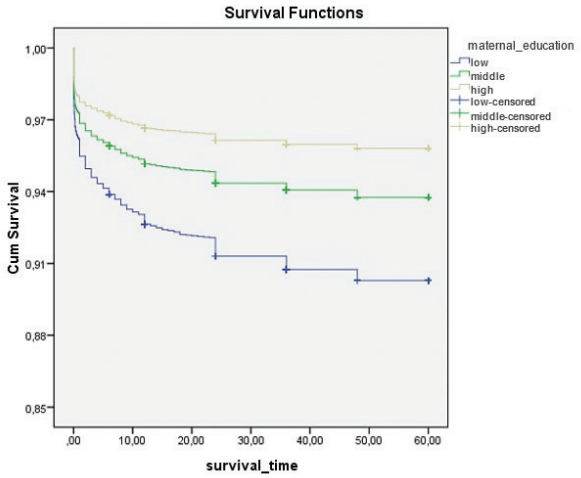

Figure 2. Kaplan-Meier curve of under-five survival in Indonesia in 2010-2015 based on maternal education status[5]

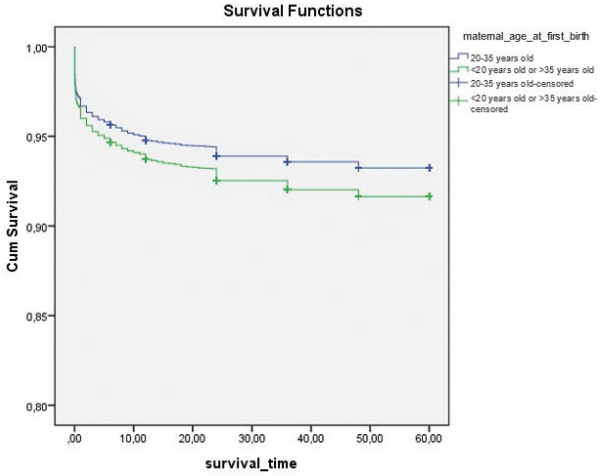

Figure 3. Percentage of under-fives in Indonesia in 2010-2015 based on maternal age at first birth[5]

under-fives with mothers aged <20 or >35 years at first birth. Kaplan-Meier test also revealed that under-fives with unemployed mothers were more likely to live longer than under-fives with employed mothers. In addition, female under-fives were more likely to live longer than male under-fives; under-fives who lived in urban areas were more likely to live longer than under-fives in rural areas; and under-fives with adequate source of drinking water were more likely to live longer than under-fives with poor source of drinking water (**Table 2**).

As shown in **Table 2**, maternal education, maternal age at first birth, maternal employment status, sex, previous birth interval, birth type, place of residence, and sanitation had significant association under-five survival. However, source of drinking water and ratio of healthcare facilities per 1000 under-fives per regency had no significant effects on under-five survival.

Kaplan-Meier test revealed that the survival curve for under-fives with previous birth interval of 24 months or more was above the survival curve for under-fives with previous birth interval of less than 24 months (**Figure 4**). Thus, under-fives with previous birth interval of 24 months or more were more likely to live longer than those with an interval of less than 24 months.

The survival curve for under-fives with single birth type was above that from those with multiple birth type. Hence, under-fives with single birth type were more likely to live longer than those with multiple birth type (**Figure 5**). **Figure 6** shows that the survival curve for under-fives with private toilets in their houses was above the survival curves for under-fives who used public toilets or had no toilet. Hence, under-fives with private toilets were more likely to live longer than under-fives who used public toilets or had no toilet.

Discussion

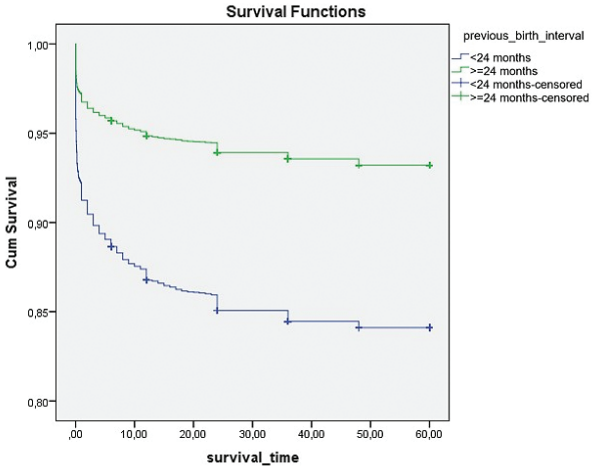

Figure 4. Percentage of under-fives in Indonesia in 2010-2015 based on previous birth interval[5]

Table 2. Parameter estimation for multilevel survival model

Variables	β	Wald	HR	P value
Individual factors				
Maternal education status				
Low	0.6648	17.58	1.9442	<0.000
Middle	0.3645	10.07	1.4398	<0.000
High*				
Maternal age at first birth				
20-35 years*				
<20 or >35 years	0.0526	2.79	1.054	<0.005
Maternal employment status				
Unemployed*				
Employed	0.1032	5.63	1.1088	<0.000
Gender				
Male	0.2404	13.93	1.2718	<0.000
Female*				
Previous birth interval				
< 24 months	0.5629	18.02	1.7558	<0.000
≥ 24 months*				
Birth type				
Single*				
Multiple	1.1879	30.11	3.2802	<0.000
Place of residence				
Rural	0.1216	5.26	1.1293	<0.000
Urban*				
Source of drinking-water				
Poor*				
Adequate	0.0356	1.95	1.0363	0.051
Sanitation				
Private toilet*				
Public toilet	0.1967	7.18	1.2173	<0.000
No toilet	0.2087	8.81	1.2321	<0.000
Contextual factor				
Ratio of healthcare facilities per 1000 under-fives per regency	-0.0014	-1.85	0.9986	0.065
β	-4.4782	-82.62	0.0114	<0.000
ln ρ	-1.2694	-154.12	-1.2694	<0.000
ρ	-3.4505		-3.4505	
Level 2 residual variable	0.1214		0.1214	

The hazard ratio showed that under-fives whose mothers had higher maternal education were less likely to die. This finding was in agreement with a previous study which showed that maternal education level had a significant effect on under-five survival.6 Educated mothers may attach a higher value to the welfare and health of children, have greater decision-making power on health-related and other matters, be less fatalistic about disease and health, be more knowledgeable about disease prevention and cure, be more innovative in the use of remedies, and be more likely to adopt new codes of behavior which improve children's health though they are not perceived as having direct consequences for health.[6]

Under-fives whose mother's age at first birth was under 20 or more than 35 years, had a higher mortality risk than under-fives whose mother's age at first birth was between 20-35 years. Similarly, a study showed that under-fives with mothers who first gave birth at either an early or late age faced a higher risk of death.[7]

The hazard ratio for maternal employment status

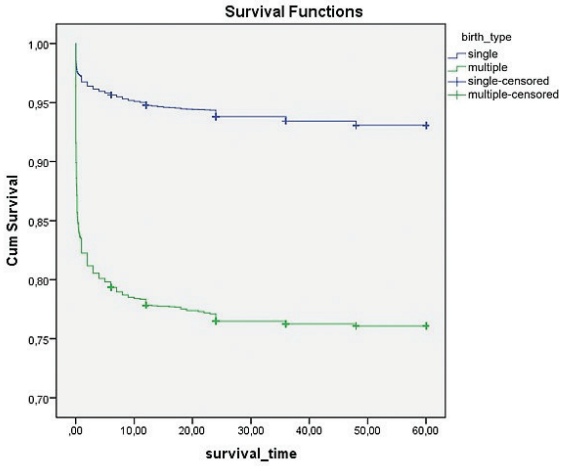

Figure 5. Percentage of under-fives in Indonesia in 2010-2015 based on birth type[5]

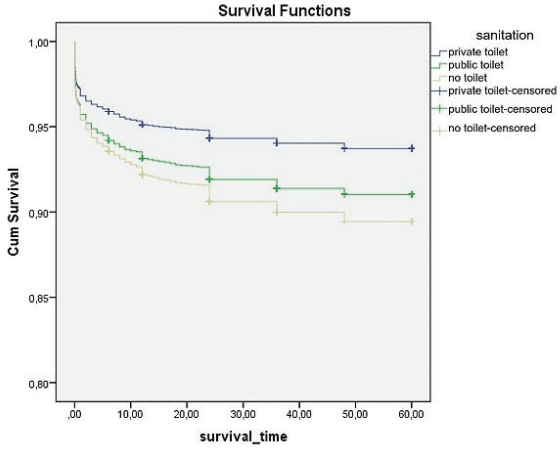

Figure 6. Percentage of under-fives in Indonesia in 2010-2015 based on sanitation[5]

showed that under-fives with employed mothers also had a higher risk of death than those with unemployed mothers, similar to a previous study.[8] Furthermore, female under-fives had lower risk of death than males. Another study had similar findings.[9] This is due to boys are vulnerable to perinatal conditions, congenital anomalies, and infectious diseases than girls.

In addition, under-fives with previous birth interval 24 months or more had lower death risk than under-fives with previous birth interval under 24 months, similar to a previous study.[10] Another study also noted that the odds of under-five mortality were reduced by longer preceding birth interval.[6] Longer birth interval could allow time for mothers' bodies to replenish nutrients, in order to be better prepared for the next pregnancy and breastfeeding.[7]

We found that multiple births had a higher death risk than single births, similar to a study conducted by Gebretsadik *et al*.[11] The twin pregnancies have an increased risk of premature birth, uneven growth and other complications. This may lead to a higher risk of mortality under five.

In addition, under-fives who lived in urban areas had lower risk of death than those in rural areas. Two studies had similar findings and reported significantly more under-five mortality in rural areas.[9,12] With regards to sanitation, under-fives whose households used public toilets or had no toilet access had a higher death risk than under-fives whose households had private toilets. A study also showed that under-fives who lived in more sanitary environments had lower death risk,[13] while another study found that good sanitation was a significant factor to decrease under-five mortality.[7]

According to the Kaplan-Meier curves and percentages, under-fives with low maternal education, maternal age at first birth <20 years or >35 years, employment, previous birth interval of <24 months, male sex, rural residence, adequate source of drinking water, and having no toilet had a higher mortality.

In conclusion, using multilevel survival analysis, individual factors affecting the survival of under-fives are maternal education, maternal age at birth of first child, maternal employment status, sex, previous birth interval, type of birth, place of residence, and sanitation. Two variables, source of drinking water and health center ratio per 1000 infants per district were not significantly associated with under-five survival. Furthermore, Indonesian government should take an action in improving female literacy and healthcare facilities and sanitation especially in rural areas.

Conflict of Interest

None declared.

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Original Article

Comparison of *Growth Diagrams Of Indonesian Children* to *2006 World Health Organization Growth Standards* in diagnosing stunting

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Abstract

Background Stunting represents a linear growth disturbance due to chronic malnutrition, recurrent infection, and inadequate psychosocial stimulation. The *2006 World Health Organization (WHO) Growth Standards* are utilized as a modality in monitoring children's growth, but to date, there has been no recommendation on use of the *Growth Diagrams of Indonesian Children* to monitor the growth of Indonesian children.

Objective To determine the proportion of stunting, the sensitivity and specificity of *Growth Diagrams of Indonesian Children* for diagnosing stunting. In addition, we aimed to compare proportions of stunting using the *2006 WHO Growth Standards* and *Growth Diagrams of Indonesian Children*.

Method A cross-sectional study was conducted in Lawe Alas District, Southeast Aceh, Indonesia. Subjects were children aged 1-59 months who fulfilled the inclusion criteria. Weight and height measurements were plotted on the *Growth Diagrams of Indonesian Children* and on the *2006 WHO Growth Standards to determine* the stature of subjects. Stunting was defined as the index Z-score for HAZ of less than -2 SD for the *2006 WHO Growth Standards*, and an HAZ index of below the 10th percentile (p10th) for the *Growth Diagrams of Indonesian Children*.

Results Of 141 subjects, 66 (46.8%) had stunting based on the *2006 WHO Growth Standards* and 51 (34.8%) had stunting based on *Growth Diagrams of Indonesian Children*. The sensitivity and specificity of the *Growth Diagrams of Indonesian Children* were 75.5% and 98.66%, respectively. Significantly more children were considered to be stunted using the *2006 WHO Growth Standards* than using the *Growth Diagrams of Indonesian Children*.

Conclusion Stunting prevalence is high in Southeast Aceh. The *Growth Diagrams of Indonesian Children* is a spesific and sensitive tool to diagnosed stunting in accordance with Indonesian children's growth patterns. **[Paediatr Indones. 2020;60:95-100; doi: http://dx.doi.org/10.14238/pi60.2.2020.95-100].**

Keywords: : *WHO Growth Standards 2006; Growth Diagrams of Indonesian Children; stunting*

Stunting is characterized by short stature, but not every child with short stature is stunted.[1] Stunting reflects a disruption of linear growth due to chronic malnutrition, recurrent infections, and inadequate psychosocial stimulation due to environmental conditions and socioeconomic status.[2,3] By definition, stunting is the index of body length by age (BAZ) or height by age (HAZ), with Z-score less than -2 standard deviations (SD).[3] Stunting that occurs before the age of 2 years is predicted to lead to adverse cognitive functions.[4] In addition to the decline of cognitive abilities,[4]

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patients with long term stunting have poor immunity, so they easily get sick, increasing their morbidity and mortality.[5]

According to the *National Basic Health Research Report (Riskesdas)*, the prevalence of stunting in Indonesian children was above 30% in four separate years (2007, 2010, 2013 and 2018). Indonesia's prevalence of stunting was 36.8% in 2007, 35.6% in 2010, 37.2% in 2013, and 30.8% in 2018.[6-9] According to the *2018 National Basic Health Research Report*, Aceh was in the top three regions with the highest stunting prevalence in Indonesia.[9]

A diagnosis of stunting can be made by several standards, including the *National Center for Health Statistics/Centers for Disease Control* (NCHS/CDC) or the *2006 World Health Organization Child Growth Standards*. The most commonly used chart in Indonesia is the *2006 WHO Growth Standards* for children under 5 years.[2] Many countries have used the *2006 WHO Growth Standards* as a modality for monitoring children's growth. However, the *2006 WHO Growth Standards* are not always appropriate for assessing child growth due to differing racial, demographic, and growth patterns among the world's nations.[10]

To date, no recommendations have been made regarding child growth curves that best represent Indonesian child growth. So, in 2006 Batubara *et al.*[11] conducted a study to create a standard growth curve for Indonesian children. However, to our knowledge, no study has compared the *Growth Diagrams of Indonesian Children* to the *2006 WHO Growth Standard Curve*, with regards to the most appropriate chart for Indonesia's health workers in daily health practices. Therefore, we aimed to assess the sensitivity and specificity of the *Growth Diagrams of Indonesian Children* in diagnosing stunting in children in the Southeast Aceh District.

Methods

This cross-sectional study was done to assess the sensitivity and specificity of the *Growth Diagrams of Indonesian Children* compared to the *2006 WHO Growth Standard*. The subject population in this study were children aged 1 to 59 months and 30 days who lived in the Village Ngkeran and Lawe Konker, Lawe Alas District, Southeast Aceh District from December 2017 to May 2018. Subjects' parents provided written informed consent. Children with malignant or autoimmune diseases, bone abnormalities, or who received long-term steroid therapy which affects linear growth were excluded. This study was approved by the Research Ethics Committee of the Universitas Sumatera Utara Medical School.

Subjects' demographic data were collected consisting of gender, age, gestational age, birth weight, and birth length. Weight measurement for young children not yet able to stand was done using a *Seca 725* baby scale, while weight measurement for children able to stand was done using a *Seca 803* footprint scale. The infants wore only underwear during the weight measurement. The scale needle was read when the child was calm and the needle stayed constant. Subjects' body lengths were measured by a *Seca 334* infant scale for those under 2 years or a *Seca 206* microtoise for children above 2 years. Subjects wore no footwear while body length/height was measured.

Anthropometric data were plotted on the *Growth Diagrams of Indonesian Children* and the *2006 WHO Growth Standards* to determine the stature of subjects. Stunting was defined as the index Z-score for HAZ of less than -2 SD for the *2006 WHO Growth Standards*,[3] and an HAZ index of below the 10th percentile ($p10^{th}$) for the *Growth Diagrams of Indonesian Children*.[11]

Univariate analysis was performed on the data to determine the distribution of subjects' characteristics. Sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio were analyzed in a 2x2 table. McNemar test was used to compare the prevalence of stunting between the two growth charts. Results with P values <0.05 were considered to be significant, with 95% confidence intervals.

Results

The characteristics of 141 study subjects are shown in **Table 1**. The stunting prevalence was 36.2% by the *Growth Diagrams of Indonesian Children* and 46.8% by the *2006 WHO Growth Standards*.

The diagnostic analysis results of the *2006 WHO Growth Standards* as the gold standard compared to the *Growth Diagrams of Indonesian Children* are

shown in **Table 2**. The *Growth Diagrams of Indonesian Children* had 75.75% sensitivity, 98.66% specificity, 98.03% positive predictive value, 82.22% negative predictive value, as well as positive likelihood ratio of 75 and negative likelihood ratio of 0.24. There was a statistically significant difference in the diagnosis of stunting using *Growth Diagrams of Indonesian Children* compared to the *2006 WHO Growth Standards* (P <0.001).

Discussion

The *2006 WHO Growth Standards* is the current growth chart used in Indonesia for children under 5 years of age. This chart was made based on data from 8,500 children from several health centers throughout the world. The study was conducted on children from various ethnic backgrounds who received exclusive breastfeeding,[3] but there were no representatives from Southeast Asian countries in the sampling of the study.[2]

The population of Indonesian children has a lower mean height than the average population of children in Europe. A previous study conducted a meta-analysis of anthropometric data in 55 countries compared to the *2006 WHO Growth Standards*. The study found that European populations had a +0.5 SD higher mean height compared to the height of the *2006 WHO Growth Standards*. Asian populations such as Saudi Arabia and India had a -0.5 SD lower mean height compared to the *2006 WHO Growth Standards*.[12] Another study stated that the German population of children less than 5 years of age had mean height at the 60th percentile in males and 62nd percentile in females.[13] Although the WHO recommends using only one type of growth chart standard, a previous study recommended that national growth chart standards be the guide for each country.[13] Consistent with this recommendation, another previuous study showed that the use of the *2006 WHO Growth Standards* led to underdiagnosed stunting in children in Australia.[14]

Table 1. Characteristics of study subjects

Characteristics	(N = 141)
Sex, n (%)	
Male	73 (51.8)
Female	68 (48.2)
Median age (range), months	17.0 (1.0-60.0)
Median weight (range), kg	9.2 (3.0-21.0)
Mean height (SD), cm	77.8 (13.68)
Median gestational age (range), weeks	39.0 (35.0-40.0)
Median birth weight (range), grams	2750.0 (2100.0-3150.0)
Median birth height (range), cm	48.0 (44.0-51.0)
Height/age by *2006 WHO Growth Standards*, n (%)	
Stunted	66 (46.8)
Not stunted	75 (53.2)
Height/age by *Growth Diagrams of Indonesian Children*, n (%)	
Stunted	51 (36.2)
Not stunted	90 (63.8)

Table 2. Analysis of the *2006 WHO Growth Standards* compared to the *Growth Diagrams of Indonesian Children*

Height/age based on *Growth Diagrams of Indonesian Children*	Height/age based on *2006 WHO Growth Standards*		Total	P value*
	Stunted (%)	Not stunted (%)		
Stunted	50 (98.1)	1 (1.9)	51 (36.2)	< 0.001
Not stunted	16 (17.8)	74 (82.2)	90 (63.8)	
Total	66 (46.8)	75 (53.2)	141 (100)	

* McNemar test

Children should be screened for stunting in order to make an early diagnosis to correct the problem. Hence, a high sensitivity diagnostic examination is needed. Moreover, positive predictive value and negative predictive value are more important than sensitivity and specificity. These values can predict the likelihood of someone suffering from a disease if the results are positive, and the likelihood of someone to be healthy if the results of the examination are negative.[15] In the comparison to the *2006 WHO Growth Standards*, the *Growth Diagrams of Indonesian Children* had sensitivity of 75.75%, specificity of 98.66%, positive predictive value of 98.03%, negative predictive value of 82.22%, positive likelihood ratio of 75, and negative likelihood ratio of 0.24. Previous study comparing the proportions of stunting based on the two growth charts, showed that stunting is often overdiagnosed with the *WHO Growth Standards* assessment.[12] To our knowledge, this is the first study to compare the accuracy of the *Growth Diagrams of Indonesian Children* as a national growth chart to the *2006 WHO Growth Standards*.

The Basic Health Research Report recorded the prevalence of national stunting in 2018 to be 30.8%.[9] Several national surveys in other countries such as Nigeria in 2018,[16] India in 2017,[17] Ethiopia in 2018,[18] Guatemala in 2019,[19] and Gabon in 2008,[20] showed that successive stunting prevalences were 30.2%, 38.4%, 41.8%, 46.5% and 30.5%, respectively. The *2006 WHO Growth Standards* were used in those studies as a reference for the classification of stunting.

A study in Argentina compared the *2006 WHO Growth Standards* to the *Argentina Pediatric Society* (APS) *Committee of Growth and Development Chart* found prevalences of stunting to be 7.9% and 5.3%, respectively.[21] The authors noted that the difference in proportion may have been due to differences in age group and cut-offs between the APS and WHO criteria. Moreover, the WHO sample was standardized to be children who were healthy, well fed according to the WHO nutrition recommendation, and growing in good socioeconomic environments which supported good growth and development. Complex statistical analysis was done to normalize the asymmetrical distribution by removing extreme values.[21] The *2006 WHO Growth Standards* also has selective drop out criteria, which means that the chart is based on ideal child growth projection, and not based on real growth data.[3]

A study reported that Indonesian boys and girls were significantly shorter than the *2006 WHO Growth Standards*.[22] In our study, there was a significant difference in the proportion of stunting based on *2006 WHO Growth Standards* (46.8%) compared to the *Growth Diagrams of Indonesian Children* (36.2%). Our results were similar to several studies in other developing countries. Another study stated that the average height population of Indonesia's children was 1.47 SD lower in males and 1.43 SD lower in females, compared to the population of children in America. The WHO has 0.5 SD as the standard for significant difference. This greatest difference is at four years of age in boys and twelve years in girls.[11] This situation may lead to lower proportions of stunting using the *Growth Diagrams of Indonesian Children* compared to the *2006 WHO Growth Standards*.

Internal and external factors play important roles in the cause of stunting. Pre-natal and post-natal factors are included in the internal risk factors that cause stunting. In addition, chronic infections in infants and children also greatly affect the occurrence of stunting.[23] Other post-natal factors that can cause stunting are chronic recurrent infections, such as chronic diarrhea and acute respiratory infection.[24] External factors including sanitation and hygiene, play an important role in preventing stunting, since poor sanitation and hygiene increase the risk of chronic infection.[25]

The limitations of this study were the small sample size, no risk factor analysis, and the population was comprised from only one region. This study also had potential risks of bias because workups on other causes of short stature, such as Patau syndrome or growth hormone deficiency, were not conducted. For such reasons, further multicenter study needs to be done with a larger number of study subjects and with a risk factor analysis for causes of stunting.

In conclusion, the prevalence of stunting in this study is high by using both of the *Growth Diagrams of Indonesian Children* and the *2006 WHO Growth Standards*. Therefore, prevalence is higher when the *2006 WHO Growth Standards* is used. The *Growth Diagrams of Indonesian Children* appear to be a solid and reliable tool to diagnose stunting in Indonesian children. Shifting to the *Growth Diagrams of Indonesian*

Children may have considerable implications for child health programmes.

Conflict of Interest

None declared.

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Original Article

Etiologies of neonatal cholestasis at a tertiary hospital in Bangladesh

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Abstract

Background Neonatal cholestasis is an important etiology of chronic liver disease in young children. It has a varied etiology. There is considerable delay in presentation and diagnosis of neonatal cholestasis in Bangladesh. Lack of awareness and knowledge among the pediatricians regarding etiological diagnosis and outcome of neonatal cholestasis is the reasons for poor outcome in major portion of cases in Bangladesh.

Objective To evaluate the etiological spectrum of neonatal cholestasis.

Methods This retrospective study was conducted at the Department of Pediatric Gastroenterology and Nutrition, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. We reviewed medical records of children who were diagnosed with neonatal cholestasis. Complete diagnostic profiles of every cases with age of presentation, male-female ratio and final diagnosis were analyzed.

Results A total of 114 children with neonatal cholestasis were evaluated. Subjects' male-female ratio was 1.92: 1.0, and mean age at hospitalization was 92.7 (SD 39.5) days. Biliary atresia was the most common etiology (47.4%), followed by idiopathic neonatal hepatitis/INH (21.9%). Other identified etiologies were, toxoplasmosis, others (syphilis, varicella-zoster, parvovirus b19), rubella, cytomegalovirus (CMV), and herpes/TORCH infection (8.61%), progressive familial intrahepatic cholestasis/PFIC (4.4%), galactosemia (4.4%), choledochal cyst (3.5%), sepsis (1.8%), urinary tract infection/UTI (1.8%), hypothyroidism (1.8%), lipid storage disease/Niemann-Pick disease (0.9%), non-syndromic paucity of interlobular bile ducts (2.67%), and Caroli's disease (0.9%).

Conclusion In Bangladesh, neonatal cholestasis cases are most often due to obstructive causes, particularly biliary atresia. Idiopathic (INH), infectious (primarily TORCH), metabolic, and endocrine causes followed in terms of frequency. **[Paediatr Indones. 2020;60:66-70; doi: http://dx.doi.org/10.14238/pi60.2.2020.66-70**].

Keywords: *neonatal cholestasis; biliary atresia; idiopathic neonatal hepatitis*

Neonatal cholestasis is defined as conjugated hyperbilirubinemia occurring in newborns within the first three months of life, due to a group of hepatobiliary disorders.[1,2] Jaundice is due to increased serum bilirubin concentration and becomes apparent in infants when the serum bilirubin level reaches >4 to 5 mg/dL.[3,4] Jaundice in the first 2 weeks of life is common, occurring in 2.4-15% of newborns.[5] Most often, this jaundice is due to unconjugated hyperbilirubinemia and resolves spontaneously,[6] but cholestatic jaundice in infancy is an uncommon and potentially serious problem that indicates hepatobiliary dysfunction.[6]

Conjugated hyperbilirubinemia in a neonate is defined as a serum direct/conjugated bilirubin concentration >1 mg/dL if the total serum bilirubin

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(TSB) is <5 mg/dL, or >20% of TSB if the TSB is ≥5 mg/dL.[7] The incidence of neonatal cholestasis is 1 in 2,500-5,000 live births.[6] Early and accurate diagnosis of neonatal cholestasis is crucial for proper management because some causes are treatable.[8] In the past, idiopathic neonatal hepatitis (INH) was the most common diagnosis, with an incidence of 1 in 4,800 to 1 in 9,000 live births.[9,10] Currently, the most commonly identifiable cause of neonatal cholestasis is biliary atresia (BA), accounting for 20-35% of neonatal cholestasis cases.[11,12] Genetic disorders and metabolic disease are also common etiologies. Congenital toxoplasmosis, rubella, cytomegalovirus, and herpes simplex (the TORCH infections) account for about 5% of cases. Differential diagnoses include obstructive conditions (biliary atresia, choledochal cyst, syndromic and non-syndromic paucity of interlobular bile ducts, inspissated bile syndrome, Caroli's disease), genetic conditions (alpha-1-antitrypsin deficiency, Alagille syndrome, progressive familial intrahepatic cholestasis/PFIC, cystic fibrosis), infectious disease (congenital TORCH infection, bacterial sepsis, urinary tract infection), metabolic conditions (bile acid synthesis defects, gestational alloimmune liver disease/neonatal hemochromatosis, galactosemia, hereditary tyrosinemia, storage diseases), and endocrine conditions (hypothyroidism, panhypopituitarism), histiocytosis, parenteral nutrition, as well as drugs.[6]

The aim of the study was to evaluate the etiological spectrum of neonatal cholestasis.

Methods

This retrospective, observational study was carried out in the Department of Pediatric Gastroenterology, BSMMU, Dhaka, Bangladesh. We reviewed the departmental register book (inpatients) from January 2017 to December 2018. Children diagnosed with neonatal cholestasis were included in this study. Diagnostic evaluations included complete blood count, liver function tests, urine for non-glucose reducing substances, urine cultures, TORCH screening, ultrasonography of the hepatobiliary system (fasting and after feeding), thyroid function tests, hepatobiliary scintigraphy, and liver biopsies. Eye evaluation was done for cataracts, chorioretinitis, cherry red spots, posterior embryotoxon, and hypoplasia of the optic discs. Patients with incomplete data were excluded from this study. A total of 114 patients were evaluated and data were entered into Microsoft Excel and analyzed by *SPSS* software. This study got ethical approval from Departemental Review Board (DRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka.

Results

A total of 114 children with neonatal cholestasis were included, 65.8% males and 34.2% females, with a 1.92:1 male: female ratio. Mean age at hospitalization was 92.7 (SD 39.5) days ranging from 23 to 270 days of age.

In the study population, an obstructive cause was most common (58.9%), followed by idiopathic (21.9%), infectious (12.2%), metabolic (5.3%), and endocrine (1.8%) causes. In terms of etiologic diagnoses, biliary atresia was most common (47.4%) followed by INH (21.9%), TORCH infection (8.6%), PFIC (4.4%), galactosemia (4.4%), choledochal cyst (3.5%), sepsis (1.8%), UTI (1.8%), hypothyroidism (1.8%), lipid storage disease/Niemann-Pick disease (0.9%), non-syndromic paucity of interlobular bile ducts (2.7%), and Caroli's disease (0.9%) (**Table 1**).

Table 1. Etiological profiles of neonatal cholestasis

Etiologi	(N=114)
Obstructive causes, n (%)	
Biliary atresia	48 (42.1)
Biliary atresia with CMV	5 (4.4)
Biliary atresia with HSV	1 (0.9)
Non-syndromic paucity of interlobular bile ducts	3 (2.7)
Choledochal cyst	4 (3.5)
Caroli's disease	1 (0.9)
PFIC	5 (4.4)
INH	25 (21.9)
Infectious cause, n (%)	7 (6)
CMV	1 (0.9)
Toxoplasma	1 (0.9)
CMV+HSV	1(0.9)
Sepsis	2 (1.8)
UTI	2 (1.8)
Metabolic causes, n (%)	
Galactosemia	5 (4.4)
Niemann-Pick disease	1 (0.9)
Endocrine cause, n (%)	
Hypothyroidism	2 (1.8)

CMV=cytomegalovirus, HSV= herpes simplex virus

Discussion

Early and accurate diagnosis is essential for good neonatal cholestasis outcomes. Prognosis of biliary atresia is dependent on the timing of operative management, as delayed diagnosis worsens the outcome.[13] In our study, the mean age at hospitalization was 92.7 (SD 39.5, range 23-270) days and the male: female ratio was 1.92: 1. Similarly, a previous study found that mean age at presentation was 105 95 days.[4] Jain et al. reported that median age at presentation was 78 (range 15-270) days and male: female ratio was 1.17: 1,14 while a study reported that mean age at presentation was 58.3 (SD 15.3, range 1-120) days and male: female ratio was 1.63:1.[15] Physicians at primary health centers in Bangladesh have little knowledge of neonatal cholestasis, so patients tend to be referred at a significant delay. Parents also prefer to consult traditional healers before coming to the tertiary centers. Furthermore, laboratory investigation facilities are not available at every center. All these factors are responsible for delayed presentation and diagnosis.

A wide variety of etiologies are responsible for neonatal cholestasis. These are broadly categorized as obstructive, metabolic, genetic, infectious, endocrine and malignant causes, as well as parenteral nutrition, and drugs.[6] We found that an obstructive cause was most common (58.81%), followed by idiopathic causes (21.9%). As full investigation facilities are not available in Bangladesh, INH contributed a sizeable portion of neonatal cholestasis in our study, as other possiblilties could not be identified. Other causes included infectious (12.21%), metabolic (5.27%), and endocrine (1.8%) causes. Most mothers and caregivers in rural areas have poor knowledge about hygiene in caring for neonates, thus infants are predisposed to neonatal sepsis. In addition, consanguinity is very common in Bangladesh, so metabolic diseases are not uncommon as an etiology of neonatal cholestasis. A consensus report on neonatal cholestasis syndrome by the *Indian Academy of Pediatrics* stated that obstructive causes were most common (41%), followed by idiopathic (30%), infectious (17%), metabolic (4%), and other (8%) causes.[16] In addition, a previous study found that obstructive causes were most common (36%), followed by idiopathic (31%), infectious (18%), metabolic (12%), and other (5.2%) causes.[17] Other studies had similar findings.14,18-20 However, Karim *et al.*[4] found that obstructive and idiopathic causes were nearly equal, and infective causes were most common. Another study found idiopathic causes in 25%, metabolic/genetic causes in 23%, biliary obstruction in 20%, parenteral nutrition in 20%, infection in 9%, and bile duct hypoplasia in 3%.[21]

Regarding specific etiologic diagnoses, we found that biliary atresia was most common (47.37%), followed by INH (21.9%), TORCH infection (8.61%), PFIC (4.4%), galactosemia (4.4%), choledochal cyst (3.5%), sepsis (1.8%), UTI (1.8%), hypothyroidism (1.8%), lipid storage disease -Niemann-Pick disease (0.87%), non-syndromic paucity of interlobular bile ducts (2.67%), and Caroli's disease (0.87%). A previous study found that neonatal cholestasis patients had biliary atresia (25.8%), INH (24.2%), TORCH infection (28.3%), urinary infection (7.2%), choledochal cyst (6.5%), hypothyroidism with CMV infection (1.6%), Down syndrome with hypothyroidism (1.6%), Crouzon syndrome with hypothyroidism (1.6%), and alpha-1 antitrypsin deficiency (1.6%).[4] A previous study noted INH in 26.0% of cases, extrahepatic biliary atresia in 25.89 %, infection in 11.47 %, TPN-associated cholestasis in 6.44 %, metabolic disease in 4.37 %, alpha-1 anti-trypsin deficiency in 4.14 %, and perinatal hypoxia/ischemia in 3.66 %. Cytomegalovirus was the most common infection identified (31.51%) and galactosemia (36.49%) was the most common metabolic disease identified.[22] In our study, we also found CMV to be the most common infection and galactosemia to be the most common metabolic etiology. See **Table 2** for a summary of etiologies from eight neonatal cholestasis studies.

In conclusion, neonatal cholestasis has a varied etiology. Delayed diagnosis may be due to late presentation at specialized centers. Obstructive causes (primarily biliary atresia) are most common, followed by idiopathic (INH), infectious, metabolic, and endocrine causes.

Table 2. Etiologies of neonatal cholestasis in different studies

Study	Location	No. of patients	BA (%)	INH (%)	Metabolic (%)	Intrahepatic ductal paucity (%)	Infectious (%)	Others (%)
Present study	Bangladesh	114	47.4	21.9	5.3	2.7	12.2	10.6
Karim B *et al.*, 2005	Bangladesh	62	25.8	24.2	1.6	3.2	35.5	8.3
Yachha SK, 2005	India	60	55	23	3	11		
Arora NK *et al.*, 2010	India	420	30	31	12	1	18	4.2
Consensus report on neonatal cholestasis syndrome, IAP	India	1008	34	30	4	3	17	12
Mieli-Vergani G *et al.*, 1989	England	50	34.7	30.5	17.4	5.6	8.7	3.1
Jain M *et al.*, 2016	India	100	41	18			34	7.7
Mahmud S *et al.*, 2016	Bangladesh	80	37.5	25	3.3	5	25.3	3.9

Conflict of Interest

None declared.

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Original Article

Fecal calprotectin and its association with functional dyspepsia in children

Jeanette Manoppo, Rizal Somali

Abstract

Background Calprotectin is a calcium-binding protein found normally in small amounts within the digestive tract. Fecal calprotectin measurement is used as a biomarker to identify digestive tract inflammation. Functional dyspepsia is one of the most common health issues in children, occurring in 3-27%, and accounts for considerable quality of life impairment and health care expenses.

Objective To determine fecal calprotectin concentration in generally healthy children as well as to assess for a possible association between fecal calprotectin and functional dyspepsia.

Methods This cross-sectional study was conducted from February to April 2019 in primary school-aged children in Manado, North Sulawesi. Subjects consisted of 38 children aged 6-12 years. Fecal calprotectin was measured in subjects' stool specimens, and considered to be normal if fecal calprotectin concentration of < 50 μg/g. Diagnosis of functional dyspepsia was defined using the parent-filled Rome IV questionnaire form. Data were analyzed with Chi-square and Phi-coefficient correlation tests.

Results Thirty-eight subjects, 22 boys and 16 girls, were grouped according to fecal calprotectin concentration (normal *vs.* elevated) and functional dyspepsia diagnosis. Mean fecal calprotectin concentration was 312.45 μg/g in the functional dyspepsia group and 20.89 μg/g in the healthy group. Elevated fecal calprotectin was found in 55.6% of the functional dyspepsia group and 10.3% of the healthy group. There was a positive correlation between fecal calprotectin elevation and functional dyspepsia (r=0.471; P=0.004).

Conclusion Current fecal calprotectin physiological cut-off level of 50 μg/g seems valid for children aged 6-12 years. Elevated fecal calprotectin is associated with functional dyspepsia in children. **[Paediatr Indones. 2020;60:71-5; doi: http://dx.doi.org/10.14238/pi60.2.2020.71-5].**

Keywords: *fecal calprotectin; immune system; functional dyspepsia*

Calprotectin is a 36 kDa calcium-binding heterocomplex protein consisting of two heavy chains and one light chain. It belongs to the S-100 protein family and is derived predominantly from neutrophils and monocytes.[1] Being resistant to enzymatic degradation, it can easily be measured in stool with a commercially available ELISA immunoassay. Due to its high sensitivity and specificity, relative simplicity, quick turnaround time, and long stability at room temperature (up to 7 days), it has been used increasingly in the diagnostic process for inflammatory bowel disease (IBD).[2] However, this test is often underappreciated as an immune defense mechanism for intestinal mucosal tissue, as it also has anti-microbial and anti-proliferative activities.[3-5] Calprotectin (*S100A8/S100A9*) has a pro-inflammatory role in innate immunity and is characterized as a damage-associated molecular

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pattern molecule (DAMP), due to its release by activated or damaged cells under conditions of cellular stress. An emerging concept of pattern recognition involves sensing of exogenous pathogen-associated molecular patterns (PAMPs) and endogenous DAMPs via the multi-ligand receptor for advanced glycation end products (RAGE) and toll-like receptors (TLRs), enabling innate immunity to achieve our primary host defense against invading microorganisms and non-specific stress factors.[6]

In accordance with their pro-inflammatory role, calprotectin is significantly overexpressed at sites of inflammation, and there is a strong correlation between its concentration and inflammation.[6] The secretion of calprotectin by phagocytes is induced when phagocytes come into contact with inflamed endothelium. Calprotectin is thought to promote inflammation via induction of pro-inflammatory chemokines, adhesion molecules (e.g., VCAM-1 and ICAM-1) and β2-integrin, thereby mediating leukocyte recruitment, adhesion, and transendothelial migration to inflamed tissue.[7] In previous studies, fecal calprotectin was shown to be a reliable predictor of mucosal healing in patients with IBD.[8,9] This further confirms the role of fecal calprotectin in the innate immune response of intestinal mucosal tissue.

Fecal calprotectin concentrations in healthy individuals have been established in several studies. In the original study, the median stool calprotectin concentration in healthy adults was 2 mg/L, and the suggested cut-off for a positive test was 10 mg/L.[10] In the newer assay, the suggested upper limit of normal was increased by a factor of five, to 50 μg/g.[11] The pediatric reference range for fecal calprotectin was established in a study of 117 healthy children (median 13.6 μg/g; 95%CI 9.9 to 19.5 μg/g). The adult cut-off level for intestinal inflammation of 50 μg/g was suggested to apply to children as well.[12]

Functional dyspepsia presents with various signs and symptoms, which are often concerning. Functional dyspepsia occurs in 3-27% of children and accounts for a considerable impact on quality of life and health care costs. It is defined by the Rome IV classification as persistent upper abdominal pain or discomfort, not related to bowel movements, and without an organic cause, that is present for at least 2 months prior to diagnosis.[13] However, these manifestations have no organic cause and require extensive diagnostics based on clinical criteria, results of physical examination, and key diagnostic studies. In practice, diagnostics of functional disorders are frequently based on exclusion of organic causes, which implies a more or less invasive approach including radiological and endoscopic studies.[14] In children, diagnosis of functional dyspepsia can be made using the validated Rome-IV questionnaire. This type of questionnaire is preferred as rapid diagnosis can be obtained without any invasive testing needed.[15]

This study aimed to determine fecal calprotectin concentration in generally healthy children as well as to assess for a possible association between fecal calprotectin and functional dyspepsia.

Methods

Children aged 6-12 years were recruited between February and April 2019. One elementary school in Manado (#30), North Sulawesi, was chosen by simple random sampling. The minimum required sample size of 38 subjects was determined with statistical power. A total of 38 subjects were collected by consecutive sampling. Inclusion criteria were children aged 6-12 years who attended elementary school #30 in Manado. Exclusion criteria were children with any medical condition which prohibited them from attending class, other known gastrointestinal and digestive system disorders, malnutrition status (body mass index < 15), taking any anti-inflammatory medication (including NSAID and corticosteroids) within the last 7 days, and children whose parents did not consent for stool collection and filling the questionnaire.

Stool specimens were collected using a spatula/stool collector and placed in clean stool containers. Specimens were sent to *ISO-9001* certified laboratory within two hours of collection. During transport, direct sunlight and sudden change in temperature were avoided. In the laboratory, fecal calprotectin was measured using an enzyme-linked immunosorbent assay/ELISA (*f*CAL® ELISA kit, BÜHLMANN Labs). Fecal calprotectin concentration < 50 μg/g were considered to be normal, and > 50 μg/g considered to be elevated.

All 38 subjects' parents filled the Rome-IV diagnostic questionnaire parent-report form for

children and adolescents (4 years of age and older) in the Indonesian language. The questionnaire was translated from English to Indonesian by a certified medical translator based in Jakarta. A functional dyspepsia diagnosis was determined using the scoring instructions that came with the questionnaire.[15]

This study was approved by the Ethics and Research Committee at Sam Ratulangi University and subject sampling was approved by the school principal and subjects' parents. Data were analyzed with *SPSS software V. 22, IBM*. Statistical correlation between groups was analyzed using Chi-square test. A P value of < 0.05 was considered to be statistically significant. Phi-coefficient correlation analysis was performed to investigate a possible association between fecal calprotectin and functional dyspepsia.

Results

A total of 38 subjects, 22 boys and 16 girls, had a mean age of 8.73 years, ranging from 6-12 years. Subjects were grouped according to their fecal calprotectin results (normal vs. elevated) and functional dyspepsia diagnoses (functional dyspepsia *vs.* healthy).

Table 1 showed fecal calprotectin concentration between these groups. Fecal calprotectin was found to be elevated in 8 subjects (21.1%), and functional dyspepsia was found in 9 subjects (23.7%). In the functional dyspepsia group, the mean and median fecal calprotectin levels were 312.45 μg/g and 167.7 μg/g, respectively. In the healthy group, the mean and median fecal calprotectin levels were 20.89 μg/g and 7.2 μg/g, respectively.

Table 1. Fecal calprotectin concentration in the functional dyspepsia and healthy groups

Fecal calprotectin concentration	Functional dyspepsia group (n=9)	Healthy group (n=29)
Elevated	5	3
Normal	4	26
Mean (SD), ug/g	312.45 (354.6)	20.89 (51.9)
Median (range), ug/g	167.7 (11.4-993.2)	7.2 (0.6-273)

In the functional dyspepsia group, fecal calprotectin was elevated in 5/9 subjects (55.6%), in comparison with only 3/29 subjects (10.3%) in the healthy group (**Figure 1**). Data analysis using Phi-coefficient correlation showed a positive correlation between elevated fecal calprotectin and functional dyspepsia ($r=0.471$; $P=0.004$).

Discussion

The aim of this study was to assess fecal calprotectin concentration in generally healthy children. Mean fecal calprotectin concentration in the healthy group was 20.89 μg/g and median was 7.2 μg/g. A previous study reported a median fecal calprotectin of 13.6 μg/g in healthy children.12 The slight difference in results might be explained by differences in ethnicity, age groups, sample handling, or laboratory reagent calibration. However, from this study result, a fecal calprotectin physiological cut-off level of 50 μg/g seems reasonable and valid for this subject group.

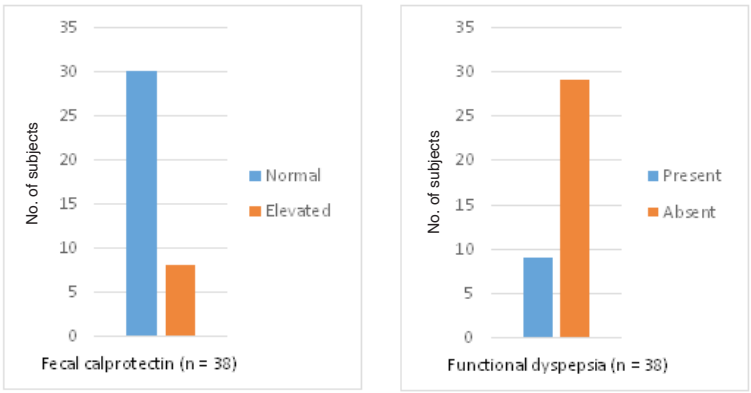

Figure 1. Fecal calprotectin and functional dyspepsia distribution of subjects

We also assessed for a possible association between fecal calprotectin and functional dyspepsia in children. Subjects with functional dyspepsia had elevated fecal calprotectin levels (mean 312.45 μg/g and median 167.7 μg/g). Moreover, Phi-coefficient correlation revealed a positive correlation between elevated fecal calprotectin and functional dyspepsia ($r=0.471$; $P=0.004$).

To our knowledge, no other studies have assessed for a possible correlation between elevated fecal calprotectin and functional dyspepsia in children. However, a study reported a significantly higher fecal calprotectin level in children with functional constipation, as compared to healthy children.[16] Our finding suggests some indications of ongoing inflammation in subjects with functional dyspepsia. Misdiagnosis is also possible, as this study determined a diagnosis of functional dyspepsia only using the parent-filled questionnaire form, without further any confirmatory tests, such as endoscopy, to rule out any organic/inflammatory causes.

Limitations of this study were the small sample size and limited diagnostic tools for diagnosing functional dyspepsia in children. Due to the fact that all of our subjects appeared to be generally healthy children, any invasive procedures to rule-out organic/inflammatory causes were not performed.

In conclusion, this study shows promising results of fecal calprotectin as a clinical test for detection of gut mucosal inflammation, as it has a significant correlation with functional dyspepsia symptoms in children. Current fecal calprotectin physiological cut-off level seems valid for children aged 6-12 years old. Elevated fecal calprotectin is associated with functional dyspepsia in children. Further studies are required to evaluate and confirm the clinical importance of fecal calprotectin as a mucosal barrier immune defense.

Conflicts of Interest

None declared.

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Original Article

Effects of probiotic on gut microbiota in children with acute diarrhea: a pilot study

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Abstract

Background Acute diarrhea is a common health problem in Indonesia. During acute diarrhea, changes in gut microbiota are marked by decrease beneficial microbes *Bifidobacterium* and *Lactobacillus*, and increased pathogenic bacteria *Enterobacter* and *Clostridium*. Such microbial imbalances are known as dysbiosis. Treatment with probiotics may help repair dysbiosis, quicken healing time, and decrease complications.

Objective To assess for dysbiosis during acute diarrhea, and determine if it can be normalized by probiotic treatment.

Methods This placebo-controlled, unblinded clinical trial was performed in Budhi Asih District Hospital, Jakarta, from January to March 2018. Twenty-four children age 6-24 months with acute diarrhea and 12 healthy children were enrolled. First fecal specimen was collected for all subjects and analyzed using non-culture real time PCR to count the population of *Lactobacillus, Bifidobacterium, Enterobacter, Clostridium*, and all bacteria. Children with diarrhea were assigned to probiotic or placebo treatment for 5 days and the second fecal specimen was analyzed two weeks after the diarrhea subsided.

Results Prior to treatment, significant higher amounts of *Lactobacillus* were observed in children with acute diarrhea than in healthy controls [median (interquartile range/IR): $1.52x10^3$ (1.22x104) *vs.* 6.87x10 (2.41x102), respectively; proportion in percentage (from total bacteria population): 0.044% *vs.* 0.003%, respectively]. However, median (IR) *Clostridium* was significantly higher in healthy controls than in children with acute diarrhea [$2.37x10^2$ ($4.64x10^3$) *vs.* 4.67 ($1.50x10^2$), respectively, with proportion of 0.01% *vs.* 0.0001%, respectively]. Children who received probio tics had significantly higher count of *Bifidobacterium* compared to the placebo group [$1.94x10^4$ ($4.97x10^4$) *vs.* $1.74x10^3$ ($2.08x10^7$), respectively, with proportion of 0.394% *vs.* 0.081%, respectively].

Conclusion This pilot study do not find evidence of dysbiosis in children with acute diarrhea. Group who received probiotic has higher *Bifidobacterium* count compared towards those who received placebo. **[Paediatr Indones. 2020;60:83-90; doi: http://dx.doi.org/10.14238/pi60.2.2020.83-90]**.

Keywords: *acute diarrhea; dysbiosis; gut microbiota; gastroenteritis; probiotic*

Diarrhea is one of the most common problems in Indonesian children, ranking third in children under 5 years' mortality, after neonatal death and pneumonia.[1] Current diarrhea treatments are based on 5 principles, also known as the 5 pillars of diarrheal management, which are: rehydration, adequate nutrition, zinc therapy, antibiotics when indicated, and education.[2] Despite these principles, probiotics are also given for the treatment of diarrhea. However, the evidence is conflicting,[3,4] and Indonesian data is lacking about the benefit of probiotics for acute diarrhea, particularly regarding microbiological changes in the gut.

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Under normal conditions, gut microbiota of children is dominated by Gram-positive anaerobic bacteria from the phyla *Firmicutes* and *Bacteroides*, such as genus *Lactobacillus* and *Bifidobacterium*. Such equilibrium is known as eubiosis.[5] Pathogenic invasion of pathogen to the intestinal mucosa damages the mucosal layer, administers toxins, and alters gut microbiota.[5,6] Gut microbiota changes occur when there is decreased commensal bacteria and increased pathogenic bacteria. This condition is known as dysbiosis.[5,6] For example, dysbiosis could occur when the commensal bacteria mentioned above decline, and Gram-negative bacteria from the *Actinobacteria* phylum (genus *Enterobacter* and *Clostridium*) increase.[6]

Probiotics are commonly used in children with diarrhea, because it can reduce the frequency of loose stools, repair stool consistency, and reduce the number of sick days.[7] On the other hand, the microbiological changes in children with acute diarrhea have not been thoroughly evaluated. There is conflicting evidence about the benefits of probiotics in acute diarrhea, particularly regarding the repair of gut microbiota composition.[8,9] To our knowledge, no study has been done in Indonesian on administration of probiotic and microbiotic changes in acute diarrhea in children. As such, we aimed to assess for dysbiosis during acute diarrhea, and evaluate microbiota alterations after probiotic administration in children.

Methods

Non-randomized, placebo-controlled, unblinded clinical trial was performed in Budhi Asih District Hospital, Jakarta. Subjects were children aged 6-24 months who visited the emergency unit, inpatient, or outpatient clinic with acute diarrhea (defined as loose stool category 5-7 in the Bristol stool chart,[10] frequency of more than 3 times a day, total duration of diarrhea less than 14 days, and no blood in stool),[11] had normal nutritional status [defined as weight for length/height between Z+2 and Z-2 (*World Health Organization 2007* curve)][12] and whose parents provided informed consent.

Children with severe malnutrition (defined as weight for length/height below Z-3 *WHO 2007* curve), persistent or chronic diarrhea, cow's milk allergy, or immune deficiency such as human immunodeficiency virus (HIV) infection, intake of high-dose steroids or chemotherapy, intake of antibiotics or probiotics within the preceding 2 months were excluded from this study. Allocation to the placebo or probiotic group was performed using consecutive sampling until the minimum required sample size was achieved. This pilot study is the first in Indonesia and we use a sample size of 12 per group (12 healthy children and 24 children with diarrhea, further divided into 12 probiotic and 12 placebo).[13]

Stool microbiota analysis was performed for *Bifidobacterium* and *Lactobacillus*, the healthy bacteria, and for *Enterobacter* and *Clostridium*, the potentially pathogenic bacteria. Total bacteria count in feces was measured as total bacteria. Absolute count is presented in copy number DNA/200 mg of feces and proportion (median of a specific bacteria divided by median total bacteria).

For normal subjects, fecal specimens were collected only once. In the diarrhea group, the first fecal specimen from the time of recruitment was referred to as T1, and second fecal specimen (2 weeks after the diarrhea subsided) was referred to as T2. The diarrhea placebo group received 20 mg zinc/day for 10 days, oral rehydration solution (ORS), and education. The probiotic group received *Probiotic Lacto-B®* from *Novell®* Pharmaceutical (content: *Lactobacillus acidophilus sp, Bifidobacterium longum sp* and *Streptococcus thermophilus sp*) twice per day for 5 days, zinc 20 mg/day for 10 days, and ORS. Diarrhea frequency, consistency, and stool characteristics were recorded every day by parents. Side effects were monitored and reported, as well as any severe adverse events (SAE). Participants in this study were not insured. Fecal specimens were delivered promptly within 2 hours stored in packaged box with cold packs to the laboratory (*Molecular Biology Laboratory*, Pediatric Gastrohepatology Division, Universitas Indonesia, Jakarta).

Fecal specimen was subjected to spin column method to isolate bacterial deoxyribonucleic acid (DNA) of *Lactobacillus, Bifidobacterium, Enterobacter, Clostridium* and all bacteria from the feces. Isolated DNA was identified using quantitative real-time polymerase chain reaction (PCR) (in total copy number DNA/200 mg of feces). Final data were presented in absolute count and proportion. Equipment used: Fast 7500 Real-time PCR (*Applied Biosystems*), computer

(*DELL optiplex 960*), mini centrifuge (*Profuge 6K*), centrifuge (*Hettich Zentrifugen*), laminar air flow cabinet (*Streamline*), splash free support base (*Micro-Amp®*), rubber base, micro pipette (*Eppendorf*), vortex (*Heidolph*), microfuge tube rack, digital scale (*AND GF-600*), freezer -20°C (*ARDO*), freezer -80°C (*New Brunswick Scientific*), nanodrop spectrophotometer (*Thermo Scientific*) and spatula.

Clinical parameters of subjects with diarrhea were converted into numerical data and compared. Statistical data analysis was performed using independent samples Kruskal-Wallis test. Changes in microbiota composition between groups were analyzed using unpaired non-parametric test Mann-Whitney or independent-samples Kruskal-Wallis test (if the data distribution was not normal). Paired data were analyzed using non-parametric T-test for normal data distribution, and Wilcoxon signed rank test for non-normal data distribution. Results were deemed significant if $P<0.05$. Statistical analyses were performed using *SPSS version 23.0* software. This study was approved by the Ethics Committee at the Universitas Indonesia Medical School.

Results

During the study period from January to March 2018, 56 patients fulfilled the inclusion criteria. However, 3 patients declined to participate in the study, and

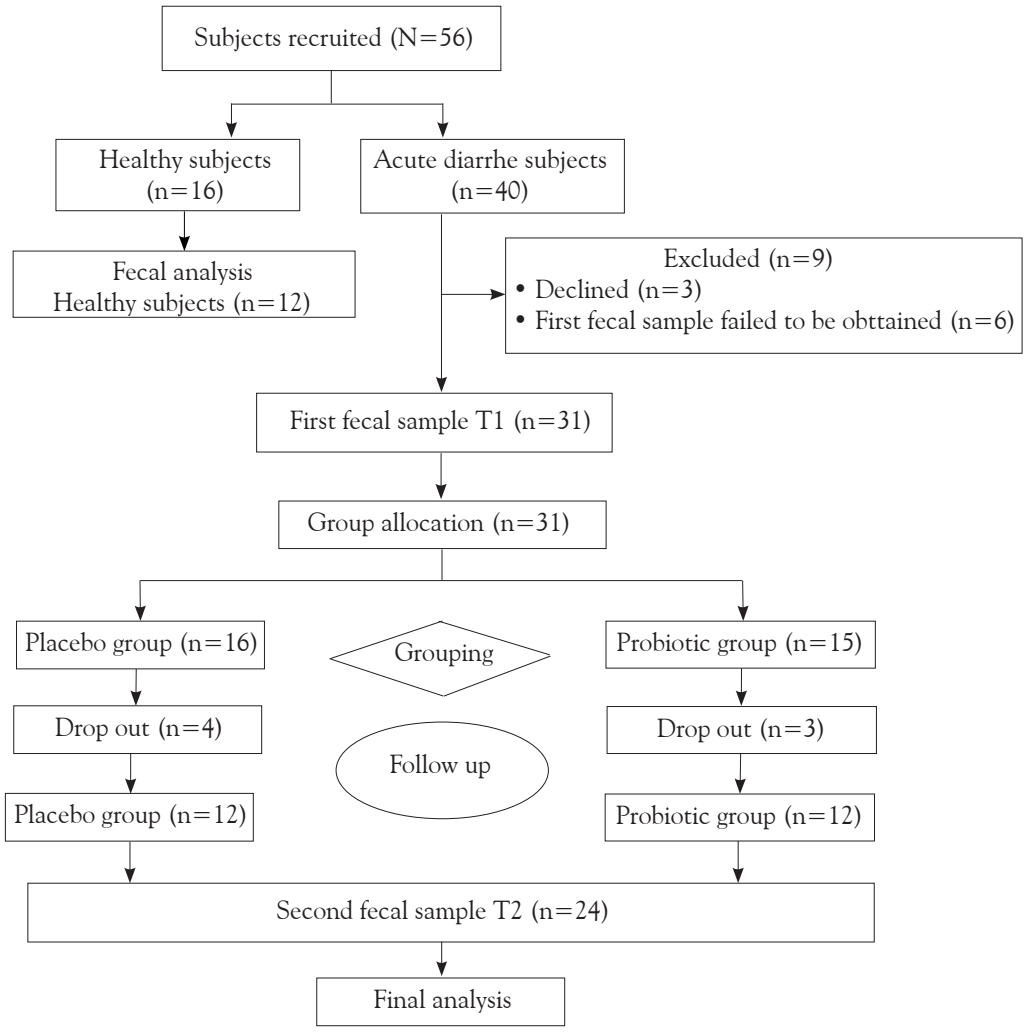

Figure 1. Study flow chart

6 patients were excluded because the initial fecal specimen was not collected. Sixteen healthy children were recruited from the children of hospital healthcare personnels. Of these, 12 children were randomly chosen for the final fecal analysis. Thirty-one patients with acute diarrhea were recruited from the outpatient department (2 patients) and the inpatient ward (29 patients). Of these, 7 patients did not provide the second fecal specimen, and therefore, were excluded from the final analysis. Hence, 24 subjects with acute diarrhea were allocated into two groups, placebo or probiotic, and included in the final analysis. A total of 36 subjects were included in the final analysis. The detailed recruitment is showed in **Figure 1**. Characteristics of subjects by group are shown in **Table 1**.

Clinical characteristics of acute diarrhea patients at the time of recruitment are provided in **Table 2**. There were no significant differences between the probiotic and placebo groups with regards to the characteristics of acute diarrhea, other accompanying symptoms, and intake of breastmilk. All the diarrhea subjects fell into the mild-to-moderate dehydration category.

Hospital length of stay [median (interquartile range)] for acute diarrhea patients in the placebo group was 4 (4) days and 3 (1) days in the probiotic group (P=0.287). Daily follow-up on loose stool frequency (defined as category 5-7 in the Bristol stool chart) was recorded (**Table 3**). After 6 days of treatment, all subjects who received probiotics showed no loose stool, whereas some subjects in the placebo group still had loose stool. (P=0.048).

Microbiota comparison between healthy and acute diarrhea subjects is provided in **Table 4**. Absolute bacteria count is presented in median (interquartile range) and proportion in %

The comparison of microbiotic composition in acute diarrhea subjects before and after intervention is shown in **Table 5**. Higher amounts of Lactobacillus were observed in children with acute diarrhea than in healthy controls [median

Table 2. Clinical characteristics of acute diarrhea at recruitment

Clinical characteristics	Diarrhea	
	Placebo group (n=12)	Probiotic group (n=12)
Median frequency of defecation (IQR)	6 (8)	8 (4)
Start of diarrhea, no. of days before*	1 (2)	2 (2)
Mucus present, n	4	3
Blood present, n	1	0
Fever present, n	8	7
Vomiting present, n	8	5
ORS treatment at home, n	4	8
Received breastmilk, n	5	4
Mild-to-moderate dehydration, n	12	12

* Results are provided in median (interquartile range). There was no significant difference (P<0.05) between characteristics in all groups.

Table 1. Characteristics of subjects

Characteristics	Healthy subjects	Diarrhea	
		Placebo group	Probiotic group
Number of subjects	12	12	12
Gender, n			
Male	8	9	8
Female	4	3	4
Age group, n			
6-12 months	7	4	3
13-18 months	2	1	3
19-24 months	3	7	6
Median age (IQR), months	12 (10)	20.5 (14)	17.5 (12)
Normal nutritional status, n	12	12	12
Median body weight (IQR), kg	9.3 (1.8)	9.2 (4.6)	10 (4.2)
Previoius rotavirus immunization, n	4	4	3

IQR=interquartile range

(IR): $1.52x10^3$ ($1.22x10^4$) *vs.* 6.87x10 ($2.41x10^2$), respectively, proportion: 0.044% *vs.* 0.003%, respectively]. However, median (IR) *Clostridium* was significantly higher in healthy controls than in children with acute diarrhea [$2.37x10^2$ ($4.64x10^3$) *vs.* 4.67 ($1.50x10^2$), respectively, with proportion of 0.01% *vs.* 0.0001%, respectively]. Children who received probiotics had significant higher count of Bifidobacterium compared to the placebo group [$1.94x10^4$ ($4.97x10^4$) *vs.* $1.74x10^3$ ($2.08x10^7$), respectively ($P<0.05$), with proportion of 0.394% *vs.* 0.081%, respectively ($P<0.05$)]. Both the placebo and probiotic groups had significantly higher *Clostridium* absolute count and proportion compared to their corresponding T1 specimens ($P<0.05$ for all).

Figure 2 shows a bar chart to better visualize the proportions of bacteria in healthy controls,

Table 3. Daily follow-up of loose stool frequency

Variables	Frequency of loose stool*						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Placebo	5 (7)	4 (5)	3 (3)	1.5 (3.8)	0 (3)	0 (3)	0 (2)
Probiotic	4.5 (4)	3.5 (3)	2.5 (1)	0 (3)	0 (2)	0 (0)	0 (0)
P value	0.294	0.305	0.253	0.251	0.281	0.048#	0.247

* Frequency of loose stool is presented in median (interquartile range).

Statistical analysis was performed using independent-samples Kruskal-Wallis test and P<0.05 was deemed significant.

Table 4. Microbiota composition in the initial fecal specimens from healthy and acute diarrhea subjects

Variables	Healthy subjects (n=12)	Acute diarrhea (n=24) First fecal sample (T1)	P value
Total bacteria	2.33×10^6 (1.05×10^7)	8.35×10^6 (1.77×10^7)	0.008^
Bifidobacterium	2.19×10^3 (3.42×10^4)	1.14×10^4 ($3.55x10^4$)	0.209
Proportion	0.094%	0.110%	0.347
Lactobacillus	6.87×10 (2.41×10^2)	1.52×10^3 (1.22×10^4)	0.012^
Proportion	0.003%	0.044%	0.034^
Enterobacter	8.79×10^3 (3.69×10^4)	9.73×10^3 (4.29×10^4)	0.023^
Proportion	0.377%	0.146%	0.388
Clostridium	2.37×10^2 (4.64×10^3)	4.67 (1.5×10^2)	0.041^
Proportion	0.01%	0.0001%	0.019^

Bacterial count is presented in median (interquartile range).

^ Significant (P<0.05) results were obtained using independent-samples Kruskal-Wallis test.

Data distribution was assessed using Shapiro-Wilk test.

Table 5. Microbiota composition in the initial fecal specimens from healthy and acute diarrhea subjects

Variables	Placebo			Probiotic			T2 placebo *vs.* probiotic P value
	T1 (n=12)	Ts (n=12)	P value	T1 (n=12)	T2 (n=12)	P value	
Total bacteria	$1.82x10^7$($4.17x10^7$)	$6.06x10^6$($5.12x10^6$)	0.019^	$7.23x10^6$($5.37x10^6$)	$3.06x10^6$($2.08x10^7$)	0.937	0.433
Bifidobacterium	$1.14x10^4$($1.66x10^4$)	$1.74x10^3$($2.08x10^7$)	0.099	$1.36x10^4$($4.33x10^4$)	$1.94x10^4$($4.97x10^4$)	0.695	0.006^
Proportion	0.110%	0.081%	0.480	0.306%	0.394%	0.308	0.015^
Lactobacillus	$5.37x10^4$($1.15x10^4$)	$3.13x10^2$($4.33x10^3$)	0.099	$5.57x10^2$($2.84x10^4$)	8.79x10 ($3.34x10^3$)	0.117	0.433
Proportion	0.078%	0.127%	0.041^	0.008%	0.025%	0.695	0.433
Enterobacter	$4.01x10^4$($1.50x10^5$)	$2.03x10^4$($2.83x10^4$)	0.136	$5.34x10^3$($8.76x10^3$)	$4.71x10^3$($1.14x10^4$)	0.754	0.084
Proportion	0.235 %	0.370 %	0.530	0.051 %	0.110 %	0.209	0.388
Clostridium	4.04 (4.34x10)	$2.49x10^2$($2.89x10^2$)	0.003^	1.78x10 ($2.36x10^2$)	$1.73x10^3$($4.07x10^3$)	0.005^	0.012^
Proportion	0%	0.003%	0.023^	0.001%	0.034 %	0.008^	0.136

Bacterial count is presented in median (interquartile range).

^ Significant (P<0.05) result was obtained using Wilcoxon signed rank test.

Data distribution was measured using Shapiro-Wilk test.

initial diarrhea fecal specimens (T1), and final fecal specimens after intervention (T2), since this was a pilot study with small sample size.

Discussion

This study investigated 36 children, 12 healthy and 24 with acute diarrhea, from ages 6-24 months in Jakarta. Other probiotic studies in Indonesia recruited 40-90 subjects in Jakarta,[14-15] Semarang,[16] Bali,[17] and Manado,[18] aged 3 months until 14 years. There were no significant differences in subjects' characteristics between the healthy and both treatment groups. Probiotic supplementation in children with acute diarrhea has been associated with shorter hospital stay, improved stool consistency, and overall health.[14-18] We noted that the probiotic group had shorter hospital stay than the placebo group (3 *vs.* 4 days, respectively), but this difference was not significant. After 6 days of treatment, all subjects who received probiotics showed no loose stool, whereas some subjects in the placebo group still had loose stool (P=0.048).

The first objective of this study was to assess acute diarrhea patients for dysbiosis, by comparing microbiota composition in healthy and diarrhea subjects (T1). Dysbiosis can be identified by changes in microbiota composition, namely increased potentially pathogenic bacteria (*Enterobacter* and *Clostridium*), and decreased commensal bacteria (*Lactobacillus* and *Bifidobacterium*).[5,6] The initial fecal specimen (T1) had significantly higher medial total bacteria, higher median *Lactobacillus*, and higher *Lactobacillus* proportion in the diarrhea group than in the healthy group. There were no significant differences between absolute count and proportion of *Bifidobacterium*. However, *Enterobacter* was significantly higher in the diarrhea group than in the healthy group, although the proportion was not significantly different. *Clostridium* counts and proportions were significantly lower in the acute diarrhea group than in the healthy group. This result was contradictory to our current hypothesis of dysbiosis in patients with diarrhea.

Two weeks after the diarrhea was cured, stool specimens were collected (T2) and analyzed for microbiota composition. The probiotic group had significantly higher *Bifidobacterium* count and proportion compared to the placebo group. Plausible explanation for this result is because the diarrhea probiotic group received probiotics which contains *Bifidobacterium longum sp*, a beneficial microbe which promote proliferation of other *Bifidobacterium* species. In addition, both the placebo and probiotic groups had significantly higher *Clostridium* absolute count

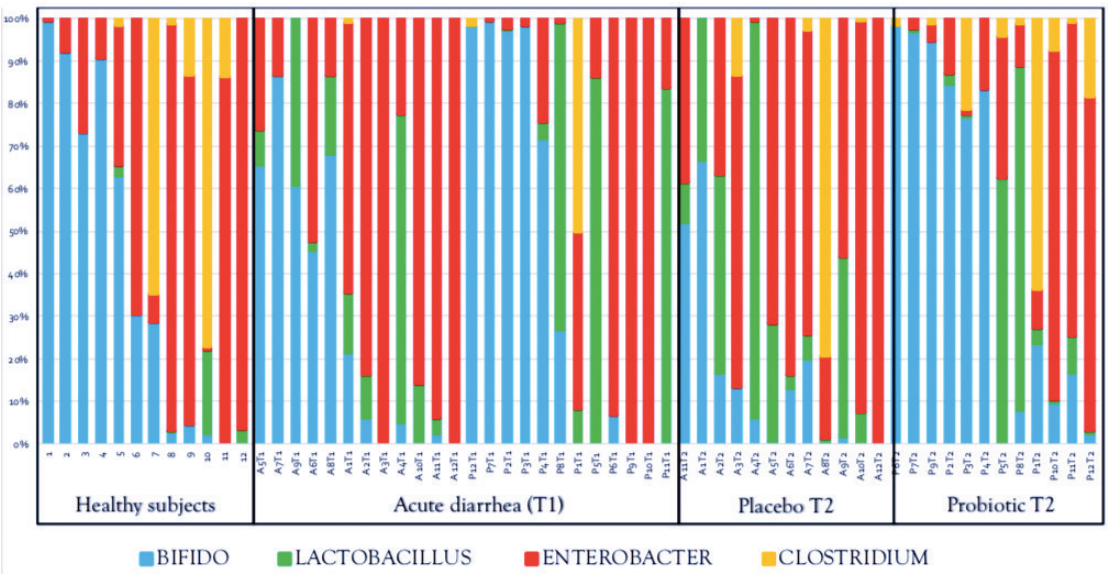

Figure 2. Bar chart of microbiota proportions in healthy subjects and acute diarrhea subjects by group, T1 and T2. This bar chart was based from proportion of each bacteria, totaling to 100%. Each bar represents bacterial composition of a specific subject in the corresponding group.

and proportion compared to their corresponding T1 specimens (**Table 5**). Based from our hypothesis that *Clostridium* rises during acute phase of diarrhea, we do not know how long this surge last, and our follow up of 2 weeks showed that *Clostridium* level is still higher than the first specimen (T1). We concluded that microbial changes after acute diarrhea might persist more than two weeks after the diarrhea ceased. A further study is needed to prove this claim. We could not prove dysbiosis in children with acute diarrhea in this study because we did not find increased potentially pathogenic bacteria (*Enterobacter* and *Clostridium*), and decreased commensal bacteria (*Lactobacillus* and *Bifidobacterium*).

To our knowledge, this is the first study to evaluate stool microbiota of children with diarrhea in Indonesia. However, we can only identify less than 1% proportion of microbiota in the stool. A previous study achieved a higher proportion (around 20%) stool microbiota identification up to the phylum level using a more advanced method known as next generation sequencing, massively parallel or deep sequencing, which was also used for the *Human Genome Project and Human Microbiome Project*.[19] Our findings are not comparable to other studies outside of Indonesia, because of differences in subjects' diets, gut profiles, and lifestyles. Furthermore, most studies had an inadequate sample size, and we could not find a single, good, randomized controlled trial (RCT) to evaluate dysbiosis in children with acute diarrhea.

There were several strengths of this study. This was the first, prospective microbiota study in Indonesian children aged 6-24 months which aimed to assess dysbiosis in acute diarrhea, as well as the effect of probiotics on dysbiosis. Also, follow-up was done 2 weeks after diarrhea ceased, and healthy comparisons were provided. The limitations of our study were the small sample size was (12 subjects for each group), the lack of randomization (consecutive sampling), the unblinded study design, and that only four genuses of gut microbiota were analyzed.

Conflict of Interest

The probiotics used for this pilot study was provided by Novell Pharmaceutical Laboratories, which also provided partial funding for this study.

Acknowledgements

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Case Report

Tuberculous pericarditis in adolescents: A case series

Winda Paramitha, Indah Kartika Murni, Eggi Arguni, Dwikisworo Setyowireni

Tuberculosis (TB) is one of the major causes of childhood mortality, especially in endemic areas. In 2013, the World Health Organization (WHO) estimated 550,000 new cases and 80,000 deaths due to TB among children. Around 70-80% of the cases were pulmonary TB, while the rest were extra-pulmonary TB.[1]

Tuberculous pericarditis accounts for only 8% of all TB cases, however, tuberculosis is the main cause of pericarditis in high-TB-burden countries, including Indonesia.[2] The mortality rate reached 17-40% and is affected by treatment adequacy.[3] Without adequate therapy, the mean life expectancy is 3.7 months, with only 20% surviving to the sixth month.[4] A 2004 study reported that successful treatment of TB in children depends on several factors, such as treatment compliance, timing and accuracy of diagnosis, concurrent human immunodeficency virus (HIV) infection and its clinical stage of disease, malnutrition, and drug resistance.[5] Adolescents and young adults are at the highest risks of having TB.[6] We report here on three cases of tuberculous pericarditis in adolescents and their outcomes following pericardiocentesis and medication. **[Paediatr Indones. 2020;60:111-6 doi: http://dx.doi.org/10.14238/pi60.2.2020.111-6]**.

Keywords: *tuberculous pericarditis; adolescents; pericardial effusion*

Case 1

A 12-year-old boy presented with pallor and fatigue. He was suspected to have malignancy and congenital heart defect at the district hospital due to right pleural effusion, which was seen by chest X-ray (**Figure 1**, left). Pleural fluid aspiration was performed; and the cytology examination revealed predominantly lymphocytes and atypical cells with large nuclei and thin cytoplasm. Tuberculin skin test was negative. Hence, he was suspected of having a malignancy and was referred to our tertiary hospital for further examinations.

Upon admission, the patient presented with fever, breathlessness, and moderate malnutrition. Chest X-ray showed pleural effusion with marked cardiomegaly (**Figure 1**, right). He was then managed as having fever on malignancy and treated empirically with intravenous antibiotics (ciprofloxacin and ampicillin). Thoracic multislice spiral CT (MSCT) performed three days later confirmed bilateral pneumonia, bilateral pleural effusion, pericardial effusion,

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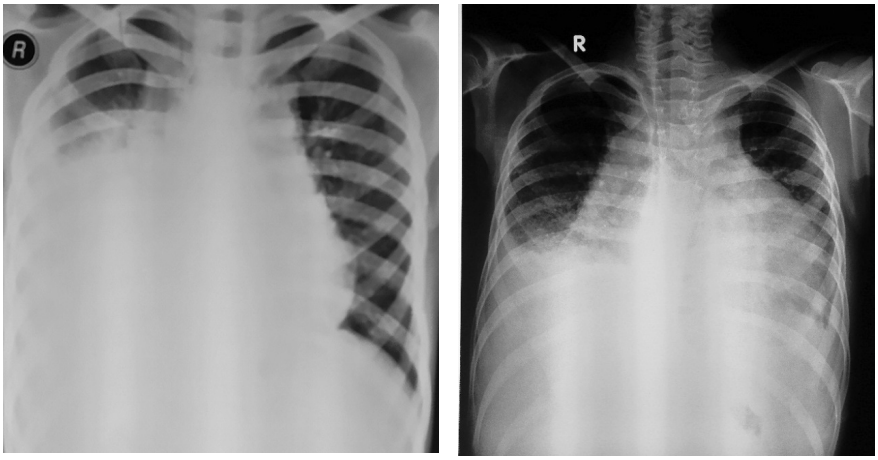

Figure 1. Chest X-rays of Case 1. Left: 2 months before admission to our hospital. Right: chest X-ray performed in our hospital.

and situs inversus (**Figure 2**). Pericardiocentesis was performed and 300 mL of serous fluid was aspirated. Pericardial catheter was inserted two days afterward, and fluid aspiration was done every 12 hours for the following 13 days. The cytology of pericardial fluid detected acid-fast bacteria, so anti-tuberculosis (anti-TB) drugs were started for tuberculous pericarditis with regimens of RHZE (rifampicin, isoniazide, pyrazinamide, and ethambutol) for 12 months, accompanied by prednisone at a dose of 1 mg/kg/day for the first two weeks, then tapered off.

After administration of anti-TB drugs, the boy's condition improved and the volume of effusion was markedly decreased. Echocardiography on the 15th day after the first pericardiocentesis showed remarkable improvement with minimal pericardial effusion. After aspirating 6 mL more of pericardial fluid, the pericardial catheter was removed. Chest X-ray after two weeks of anti-TB drugs confirmed the clinical improvement, hence, the patient was discharged on the next day. Due to the co-existence of pneumonia, the child was sent home with cloxacillin for two weeks, in addition to the anti-TB drugs. Echocardiography was performed one week after discharge at the outpatient clinic. It revealed moderate pericardial effusion. The anti-TB regimen was continued.

The patient completed the 12-month anti-TB regimen. Echocardiography at the end of the treatment revealed no pericardial effusion. No apparent complications such as cardiac tamponade or constrictive pericarditis occurred during our observation.

Case 2

Our second patient (a 17-year-old boy), was initially a referral case from a district hospital, with massive pericardial effusion. The patient mainly complained about a non-productive cough and breathlessness which had started around two weeks before hospitalization. The patient was diagnosed with acute pharyngitis by his general practitioner. However, heart enlargement was found on chest X-ray. Two weeks later, due to worsening symptoms, the patient was brought to the emergency room (ER) at a district hospital. Urgent echocardiography was performed which revealed massive pericardial effusion, hence, the patient was referred to our hospital (**Figure 3**). In our ER, emergency pericardiocentesis was performed and 1,150 mL of serohemorrhagic fluid was aspirated from the pericardial cavity. Routine pericardiocentesis was performed every 6 hours. Pericardial fluid analysis showed high protein with cellularity, predominantly lymphocytes. Pericarditis with pericardial effusion caused by non-specific bacterial infection and tuberculosis was suspected. The patient was treated empirically with antibiotics. During hospital care, routine pericardiocentesis yielded fluctuative volumes of pericardial fluid. Chest X-ray on the seventh day of care still suggested massive pericardial effusion (**Figure 4**, left). Sputum culture revealed the growth of Enterobacter cloacae and acid-fast bacilli (AFB) were detected in sputum staining. On the 8th day of care, adenosine deaminase

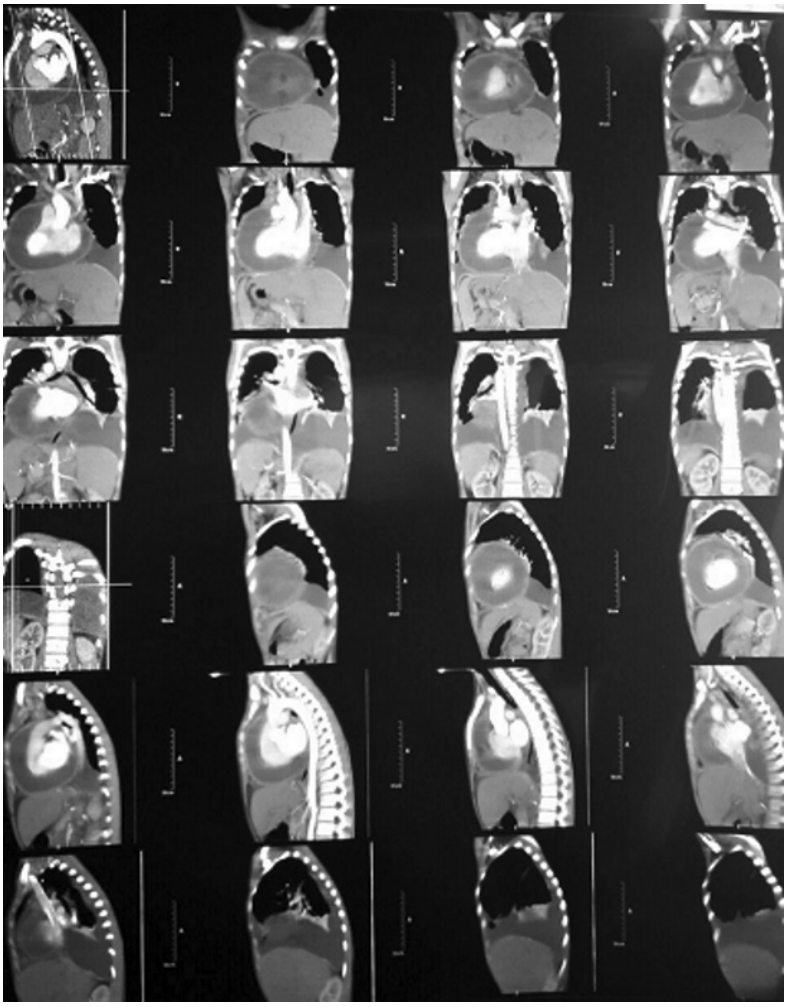

Figure 2. Thoracic MSCT of Case 1

(ADA) test resulted in of 90.1 U/L. GeneXpert assay detected Mycobacterium tuberculosis DNA in sputum. The patient was then treated for tuberculous pericarditis. Anti-TB treatment was started with the RHZE regimen. Corticosteroids were added at 1 mg/kg body weight (BW)/day for 2 weeks, then tapered off. Other comorbidities found were moderate malnutrition, hypoalbuminemia, electrolyte imbalance, and pneumonia caused by *Enterobacter cloacae* and *Candida tropicalis*. On the follow-up, the patient showed signs and symptoms of hemodynamic failure and emergency pericardiocentesis was performed. However, only 29 mL of fluid was extracted. Pericardial window was then performed on the 16th day of care, and after 27 days of hospitalization, the patient was sent home with a substantially improved condition (**Figure 4**, right).

Case 3

A 17-year-old girl initially complained of fever for 14 days prior to admission, accompanied by a 10 kg weight loss in one month. The patient was brought to a public hospital one week later due to additional complaints of breathlessness, vomiting, and abdominal pain. No additional abnormal breath or heart sounds were heard on chest auscultation. A chest X-ray was performed as indicated, with slight cardiomegaly (**Figure 5**, left). The electrocardiography finding was only sinus tachycardia and laboratory results

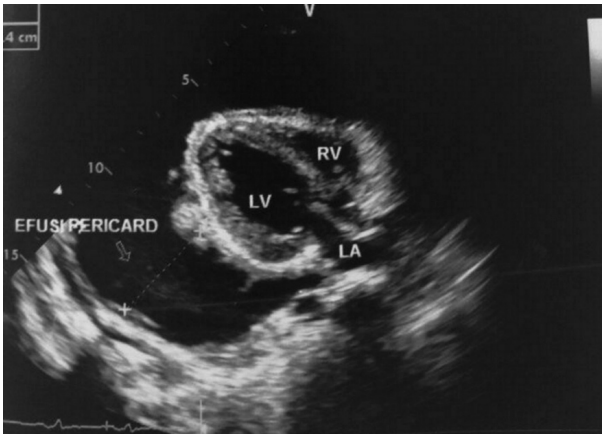

Figure 3. Case 2. Echocardiography at the district hospital revealed massive pericardial effusion

showed low hemoglobin level (7.7 g/dL). Abdominal ultrasonography (USG) revealed cystitis, ascites, and bilateral pleural effusion. The pleural effusion was suspected to be caused by either tuberculosis or non-specific bacterial infection. On the sixth day of care, due to unremarkable improvement of her condition, chest X-ray was re-performed, which revealed cardiomegaly with a water bottle shape and left pleural effusion (**Figure 5**, right). Urgent echocardiography showed massive circumferential pericardial effusion with signs of impending tamponade. The patient was referred to our tertiary hospital for further management.

The patient presented to our ER with tachycardia, tachypnea, normal blood pressure, muffled heart sounds, and hepatomegaly with low voltage ECG. Echocardiography revealed massive pericardial effusion, right ventricular collapse, right atrial collapse, and swinging heart. Needle pericardiocentesis was performed and 460 mL xanthochromic fluid was extracted. A pericardial catheter was inserted and empirical antibiotics were administered. Fluid analysis revealed exudative fluid with polymorphonuclear dominancy. Cytologic examination showed suppurative chronic inflammation with no malignant cells, but plenty of polymorphonuclear and mononuclear cells. No Mycobacterium tuberculosis or other bacteria were cultured from the pericardial fluid. No autoimmune marker was positive from the blood. However, ADA examination showed an elevated value of 52 U/L. The patient was then managed for massive pericardial effusion due to tuberculous pericarditis and was treated with anti-TB medication. Other comorbidities were moderate malnutrition and iron deficiency anemia. The patient showed remarkable improvement after treatment and was discharged after ten days of hospitalization.

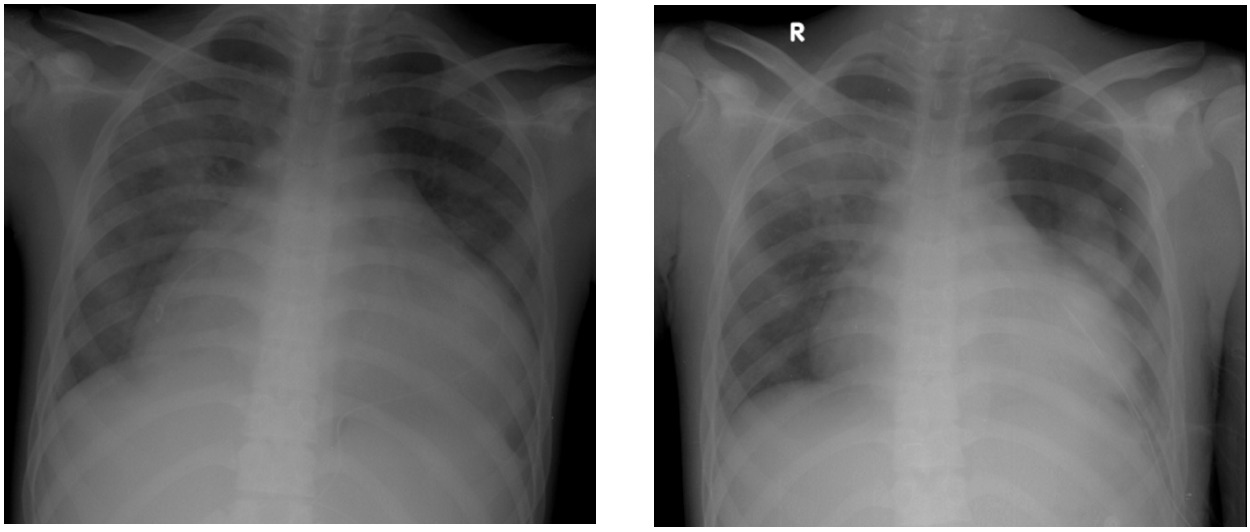

Figure 4. Case 2. Left: Chest X-ray at our tertiary hospital showed a water bottle shape indicating a massive pericardial effusion. Right: Chest X-ray on the 16th day of care after pericardial window showed marked improvement of effusion.

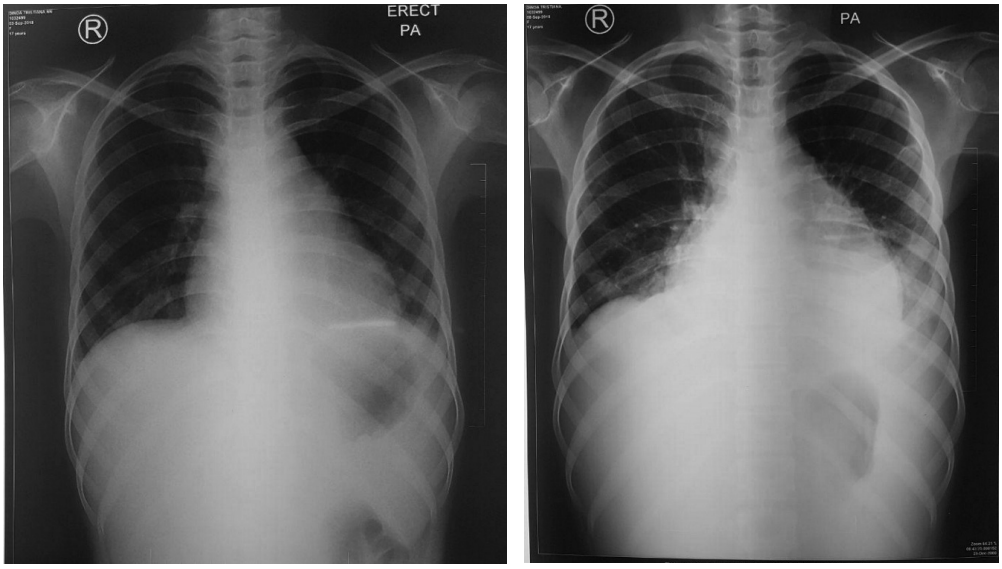

Figure 5. Case 3, Left: Chest X-ray on first day of admission at previous hospital. No abnormality was found except slight cardiomegaly with cardiothoracic ratio 0.56. Right: Chest X-ray on the sixth day of care. Cardiomegaly with water bottle shape and left pleural effusion.

Discussion

Tuberculous pericarditis is a rare form of extra-pulmonary TB, with less than a tenth of all TB cases.[7] Tuberculous pericarditis may occur due to spreading of infection from the mediastinal lymph nodes, lung, vertebrae, sternum, or miliary dissemination.[8] Based on Wallgren's timetable of primary TB, tuberculous pericarditis that arises from miliary TB may occur approximately 12 months after the primary infection.[9] Primary infection remains undiagnosed in the majority of cases, as symptoms are mild, non-specific, and usually self-resolving. The timing of primary TB and the sources of infection in our cases were unclear. Age and social environment might be considered as the most influential factors, since all our cases were adolescents. Adolescents were reported to be the most vulnerable population to contracting TB.[6]

All our cases had different signs and symptoms at the onset of illness, which commonly were not specific and might mimic other diseases. However, as the disease progressed, all developed fever and breathlessness. The major similarity of our cases was pericardial effusion as the main finding on echocardiography. Pericardial effusion is the most common manifestation of tuberculous percarditis (79.5%), followed by constrictive pericarditis with effusion (15.1%), and constrictive pericarditis without effusion (5.4%).[8] The most frequent initial symptoms are fever and breathlessness (73-97% and 80-88%, respectively), whereas pericardial rub can be found in 37-84% of cases.[8]

Tuberculosis is not the only cause of pericarditis, however, it is the most common cause of pericarditis in developing countries.[2] A definitive diagnosis of tuberculous pericarditis is made if acid-fast bacilli is found in pericardial fluid or tissue. Probable diagnosis is made if at least one of the following three conditions is found: (1) pericardial effusion with confirmed TB in other organs, (2) exudative lymphocytic effusion with increased ADA level, and (3) treatment response to anti-tuberculosis drugs.[7] Acid-fast bacilli were detected in our first and second cases, therefore, a definitive diagnosis could be made. The diagnoses were also supported by the increased ADA >30 U/L in pericardial fluid, which has sensitivity of 94% and specificity of 68% in establishing Mycobacterium tuberculosis as the cause of effusion.8 The third case showed only increased ADA, however, the patient was still managed for tuberculous pericarditis due to not finding any other possible cause of effusion. After the initiation of anti-tuberculosis treatment,

patient showed rapid improvement and discharged 10 days after.

Although all cases presented with effusion, each case showed different volumes and durations of fluid accumulation. Pericardiocentesis was performed in all cases, followed by insertion of pericardial catheter. Upon initial presentation, the largest volume of pericardial fluid was aspirated from the second case (1,150 mL). However, no tamponade signs were found. On the other hand, the third case suffered from impending tamponade before 460 mL of fluid was aspirated from her pericardial cavity. The risk of cardiac tamponade depends on the amount and duration of fluid accumulation. Acute accumulation of more than 100 mL of fluid can induce cardiac tamponade, while chronic accumulation can reach 2,000 mL of fluid without interfering with cardiac output.[10]

Anti-tuberculosis treatments were started in all cases during the hospitalization period. A 2004 study reported that successful treatment of TB in children depends on several factors, including therapy compliance, timing and precision of diagnosis, co-infection of HIV and its clinical stage, malnutrition, and drug resistance.[5] Our first and third cases presented with moderate malnutrition as the comorbidity, and none of them suffered from HIV co-infection. Corticosteroids treatment were also given to reduce the inflammatory reaction that eventually lead to tissue damage. A double blind placebo-controlled study in patients with tuberculous constrictive pericarditis found a more rapid improvement and lower mortality rate in prednisolone group compared to placebo.[11] A meta analysis witheld in 2017 also found that corticosteroids decreased the risk of all-cause mortality [risk ratio (RR) 0.80, 95% confidence interval 0.59 to 1.09] and the need for repeat pericardiocentesis (RR 0.85, 95% CI 0.70 to 1.04) in HIV seronegative patients with tuberculous pericarditis.[12] Eventually all our cases improved with combination of anti-TB treatment and corticosteroid despite all the existing comorbidities, probably also due to high treatment compliance.

In conclusion, adolescents and young adults are the most vulnerable population to contracting tuberculosis. Tuberculous pericarditis may mimic other diseases at its initial presentation. Timing of diagnosis, pericardiocentesis, and anti-TB treatment compliance are major factors in determining outcomes.

Conflict of interest

None declared.

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Original Article

Serum zinc level and prognosis of neonatal sepsis

Chaliza Adnan, I Wayan Dharma Artana, Ketut Suarta, I Gusti Lanang Sidiartha, I Wayan Gustawan, Ni Putu Veny Kartika Yantie

Abstract

Background The prognosis of neonatal sepsis can be influenced by various risk factors, one of which is a deficiency of zinc micronutrient substances.

Objective To assess for a potential association between serum zinc level and prognosis of infants with early-onset neonatal sepsis (EONS).

Methods This prospective cohort study was done in neonates with clinical EONS from September 2017 until December 2018. Serum zinc level was measured on the first day of diagnosis and prognosis was assessed on the fourth day. The association between serum zinc levels and prognosis of EONS was analyzed by Chi-square test and logistic regression with adjustment for confounding variables.

Results A total of 70 subjects were divided into two groups based on their serum zinc levels. A cut-off point of 75 ug/dL was used based on area under the curve (sensitivity 91.2% and specificity 93.7%), with accuracy 97.8% (95%CI 0.943 to 1.000; P=0.0001). Subjects with low zinc level had a 16.8 times greater risk compared to subjects with high serum zinc (RR=16.81; 95% CI 4.35 to 65.02; P <0.0001). Multivariate analysis revealed that subjects with low serum zinc levels had 203.7 times greater risk of worsening than subjects who had a higher serum zinc level (RR 203.72; 95% CI 26.79 to 1549.17; P <0.0001). Covariates such as male sex, low gestational age (<37 weeks), low birth weight (<2,500 grams), asphyxia, Caesarean section delivery, and the presence of comorbidities did not have significant associations with outcomes of EONS (P >0.05).

Conclusion Serum zinc level is associated with prognosis of early onset neonatal sepsis, with a cut-off of 75 μg/dL. The high level of serum zinc associates with a better prognosis. **[Paediatr Indones. 2020;60:37-42; doi: http://dx.doi.org/10.14238/pi60.1.2020.37-42**].

Keywords: *serum zinc levels; neonatal sepsis; prognosis; early-onset neonatal sepsis*

Neonatal sepsis is a major problem that requires special attention in the field of neonatal care because of its high prevalence and mortality. The diagnosis and management of neonatal sepsis are often late and various risk factors aggravate the condition. One of these factors is a neonatal deficiency in zinc micronutrient substances which may lead to poor prognoses.

Sanglah Hospital, Denpasar, Bali, the referral hospital on the island of Bali, had 458 suspected cases of sepsis in 2008-2009, and a prevalence of neonatal sepsis of 5.3% in 2004.[1] The incidence of neonatal sepsis in 2010 was 5.0% of patients treated. Based on preliminary data obtained from the Sanglah Hospital annual reports from 2013 and 2014, the prevalences of neonatal sepsis treated in the Neonatology Ward were 170 (15.8%) and 225 (22.0%), respectively. The mortality rate at Sanglah Hospital in 2010 was 30.4%, which means that even though the incidence of treated sepsis was low (5 per 100 patients), a

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high mortality rate of 30 out of 100 patients treated occurred.[2]

The time of sepsis onset can be a risk factor for poor prognosis, as in early-onset neonatal sepsis (EONS), which is sepsis occurring at under 72 hours of age, compared to late-onset neonatal sepsis (LONS), occurring at over 72 hours of age. Early onset neonatal sepsis is the most common cause of morbidity and mortality in premature infants.[3,4] Differences in etiologic agents have a bearing on the prognosis and long-term complications in septic newborns. In addition, deficiencies of micronutrients in the body, such as iron (Fe), copper (Cu), vitamin A, zinc, and other minerals, can aggravate sepsis. Zinc can affect prognosis in neonatal sepsis, as low zinc levels in septic neonates were noted to have a worse prognosis.[5] Various studies on zinc levels in neonates reported the average serum zinc level of healthy neonates was 75.0–98.0 μg/dL,[6] while serum zinc deficiency was considered to be <65.0 μg/dL.[7] A previous study noted decreased serum zinc levels in neonatal sepsis, which worsened in more severe sepsis.[8]

A study found that mean serum zinc levels in children with clinical symptoms of infection [45.0 (SD 13.7) μg/dL] differed from those without clinical symptoms of infection [49.0 (SD 13.7) μg/dL], but the difference was not signficant.[9] In another study, serum zinc levels in children infected with positive laboratory results (increased CRP or leukocytosis) were significantly lower compared with patients with negative laboratory results.[10]

A study showed that serum zinc levels could be used as an indicator of prognostic outcome in neonatal sepsis, in a study of 41 neonatal sepsis cases. Among the lower serum zinc group, 75.0% of septic neonates worsened, while among normal or higher serum zinc groups, only 6.9% of cases had deteriorating sepsis outcomes.[11] This study aimed to assess for a potential association between serum zinc level and prognosis of infants with early-onset neonatal sepsis (EONS).

Methods

This was a prospective cohort design study conducted in the Neonatology Ward at Sanglah Hospital, Denpasar, Bali, from September 2017 until December 2018. The inclusion criteria were neonates with clinically EONS, gestational age >32 weeks, birth weight >1,500 grams, and whose parents provided written informed consent.

The exclusion criteria were neonates with major congenital anomalies, gastrointestinal infection/disorders, multiorgan failure, or malignancy. We used a consecutive sampling method. The required minimum sample size was based on unpaired, categorical, comparative analytics, with alpha 0.05 and power 0.8, and calculated to be 33 subjects per group. Serum zinc levels was measured in venous blood specimens when clinically EONS was diagnosed. The zinc measurement was carried in the laboratory, using the inductively coupled plasma-mass-spectrometry (ICP-MS) method. Zinc levels were divided into high and low, based on the cut-off values obtained from the area under the curve (AUC) analysis. The high serum zinc level if it was above the cut off value and the low serum zinc level if it was below the cut off value. Serum zinc levels are expressed in units of ug/dL.

Clinically EONS was defined as a condition in which signs and symptoms of neonatal sepsis were found based on clinical and laboratory results (possible/probable sepsis). The clinical signs and symptoms consisted of lethargy, convulsions, whimpering cry, high pitch cry, weak suction reflexes, convex crown, hypotonic, cyanotic/pale/yellow skin, cold acral, sclerema, edema, abnormal temperature, abnormal breathing rate, abnormal pulse rate, vomiting, abdominal distension, splenomegaly, decrease of urine volume, bleeding, and shock. The laboratory results including IT ratio value >0.2, total number of PMN cells <1800/mm^3 or >5400/mm^3, leukocyte count <5000 or >30000/mm^3, and platelet count <150000 or >360000/mm^3. If there were only 3 clinical symptoms out of 6 clinical signs and symptoms groups, the subject was classified as possible sepsis. If the subject had 3 clinical signs and symptoms with laboratory abnormalities, he/she was categorized as propable sepsis.

Prognosis of EONS was defined as neonatal sepsis outcomes on the 4th fourth day of treatment. The good outcomes was defined as an improvement of clinical and laboratory findings, while the bad outcome was the worsening of clinical and laboratory findings. Data were analyzed using *SPSS software*. Data are presented in narrative and table format. Serum zinc cut-off point was defined using the receiver

operating characteristics (ROC) curve. Bivariate analysis was done by Chi-square test and multivariate analysis by logistic regression analysis. The level of significance was based on P values <0.05. This study was approved by the Research Ethics Committee of Universitas Udayana Medical School/Sanglah Hospital, Denpasar.

Results

There were 75 neonates with clinically EONS during the study period. Three neonates were excluded because of major congenital anomalies and two for necrotizing enterocolitis. The total subjects comprised of 70 neonates. The subjects underwent venous blood sampling for serum zinc examination on the day of diagnosis. All subjects were followed until the fourth day after diagnosis, at which time they were assessed for EONS prognosis.

Characteristics of subjects are shown in **Table 1**. Serum zinc levels <75 μg/dL were found in 33 subjects (47.1%) and serum zinc levels >75 μg/dL were found in 37 subjects (52.9%). The majority of the subjects (62.9%) were male, with full term gestational age (61.4%) and had normal birth weight (52.9%). The most common comorbidity was neonatal pneumonia (41.4%). The association between serum zinc levels and EONS prognosis was analyzed with Chi-square test. Subjects were categorized into two groups with a serum zinc cut-off point of 75 ug/dL based on AUC, with 91.2% sensitivity, 93.7% specificity, and 97.8% accuracy (95%CI 0.943% to1.000%; P=0.0001).

Bivariate analysis revealed that subjects with serum zinc level <75 μg/dL had a 16.8 times greater

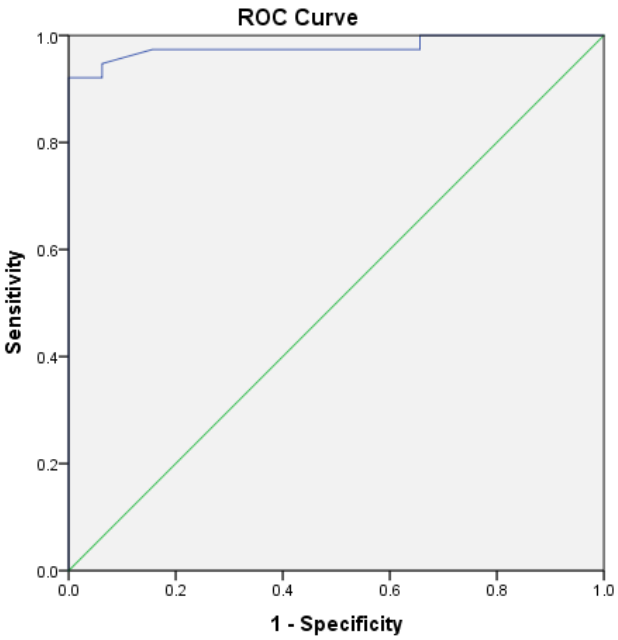

Figure1. Receiver operating characteristic (ROC) curve of serum zinc level

Table 1. Characteristics of subjects

Characteristics	Serum zinc level (N=70)	
	<75 μg/dL (n=33)	>75 μg/dL (n=37)
Gender, n		
Male	20	24
Female	13	13
Gestational age, n		
32- 36 weeks	11	16
>37 weeks	22	21
Birth weight, n		
1,500-2,499 grams	15	18
>2,500 grams	18	19
Asphyxia, n		
Yes	7	14
No	26	23
Birth method, n		
Caesarean section	9	11
Vaginal	24	26
Comorbidities		
Neonatal pneumonia	16	13
Hyaline membrane disease	4	6
Congenital heart disease	6	4
Intracranial bleeding	9	2
Neonatal jaundice	5	6
Hypoxic ischemic encephalopathy	1	4
Chorioamnionitis, n		
Yes	10	9
No	23	28
Blood smear, n		
Normal	21	27
Infection	12	10
White blood cells		
5,000-30,000/ mm^3	11	18
<5,000 or >30,000/ mm^3	22	19
Platelet count		
150,000-360,000/ mm^3	24	25
<150,000 or >360,000/ mm^3	9	12
IT ratio		
<0.2	13	21
>0.2	20	16

risk of deterioration compared to subjects with serum zinc level >75 μg/dL (RR=16.81; 95%CI 4.35 to 65.02; P <0.0001) (**Table 2**). **Table 3** shows the multivariate analysis of various potential contributing factors including serum zinc levels and EONS prognosis. Subjects with low serum zinc levels had a 203.7 times greater risk of worsening prognosis than subjects with high serum zinc level (RR=203.72; 95%CI 26.79 to 1549.18; P <0.0001). Furthermore, covariates such as male sex, low gestational age (<37 weeks), low birth weight (<2,500 grams), asphyxia, Caesarean section, and the presence of comorbidities, did not have significant associations with poor prognostic outcomes from EONS.

Discussion

In our study, zinc levels had a significant relationship with EONS prognosis. The ROC curve revealed that zinc level can be a useful predictor of outcomes in EONS cases, with an AUC value that was close to 1 (97.8%; 95%CI 0.943 to 1.000; P=0.0001). The serum zinc cut-off value was 75 μg/dL, with 91.2% sensitivity and 93.7% specificity. A previous study on 41 neonates with sepsis reported an AUC value of 0.93 (P<0.001) as well as a serum zinc cut-off value of 192.5 μg/dL based on ROC analysis, with 81.8% sensitivity and 90.0% specificity. Neonates with serum zinc level <192.5 μg/dL had a 8.18 times greater risk for worsening sepsis compared to those with higher zinc levels.[11] The different serum zinc levels, and consequently, cut-off points found in our study and that of previous study could be attributed to different inclusion criteria. The previous study used a sample of neonates with gestational age and birth weight greater than ours, and included full term babies with normal birth weight.[11]

The bivariate analysis showed that the serum zinc level in the poor prognosis group was significantly lower compared to the group that experienced improvement. Subjects with serum zinc level <75 μg/dL had a 16.8 times greater risk of deterioration compared to subjects with serum zinc level >75 μg/dL (RR 16.81; 95%CI 4.35 to 65.02; P <0.0001). Similarly, a study found lower zinc levels in conditions of severe sepsis in children aged 1-11 years.[9] Our multivariate analysis showed that subjects with low serum zinc level had a 203.7 times greater risk of worsening than subjects who had higher serum zinc level (RR 203.72; 95%CI 26.79 to 1549.17; P <0.0001).

Another study found that neonates with greater gestational age and weight tended to have higher serum zinc levels.[12] The serum zinc level obtained in each study can be different, this is also due to the influence of nutritional status, population, and techniques in taking and processing blood samples in

Table 2. Bivariate analysis of serum zinc level and EONS prognosis

Variables	Prognosis		RR	95%CI	P value
	Poor	Good			
Serum zinc level					
<75 ug/dL (n=33)	30	3	16.81	4.35 to 65.02	<0.0001
>75 ug/dL (n=37)	2	35			

Table 3. Multivariate analysis of serum zinc levels and EONS prognosis

Variables	Exp (B)	95% CI	P value
Serum zinc <75 ug/dL	203.72	26.79 to 1549.18	<0.0001
Male sex	1.39	0.19 to 9.94	0.744
Gestational age <37 weeks	0.83	0.05 to 14.15	0.899
Low birth weight	1.31	0.09 to 20.02	0.847
Asphyxia	1.34	0.16 to 11.01	0.783
Caesarean section	0.51	0.06 to 4.10	0.526
Comorbidities	2.24	0.23 to 22.20	0.490

each laboratory. However, there were no data showing normal zinc levels for gestational age and infant birth weight.

A study used copper/zinc ratios in EONS patients and showed that the Cu/Zn ratio in infected neonates was relatively higher, indicating that zinc levels acting as denominators in the ratio formula tended to decrease. Statistical tests showed that plasma Cu levels in neonates with sepsis were not significantly different from that of controls (neonates without sepsis) (P=0.177), while zinc levels were significantly lower than that of controls (neonates without sepsis) (P <0.001). They also found that plasma zinc concentrations did not have significant correlations with other variables such as gender, gestational age, or birth weight in neonates with a diagnosis of EONS.[13]

We also found that low serum zinc was significantly associated with the outcome of sepsis. On multivariate analysis, covariates such as male sex, <37 weeks gestational age, birth weight <2,500 grams, asphyxia, Caesarean section, and the presence of comorbidities, did not have significant associations with outcomes from EONS (P >0.05). Since only zinc level had an association with worsening sepsis prognosis in our study, it was likely not influenced by variables shown to be not significant by multivariate analysis, and the similar proportions between these groups.

The results of multivariate regression analysis using the independent variable selection model and its effect on the dependent variable, resulted in an R-squared value of 78%. The remaining 22% would account for other variables that were not analyzed, such as other factors that can affect the prognosis of sepsis. Such factors may include the lack of other micronutrient substances such as iron (Fe), copper (Cu), vitamin A, and other minerals not examined in this study and also the etiologic agents was not analyzed.

A previous study showed that mortality nine months or more after sepsis diagnosis among neonates with zinc levels below 70 μg/dL was 86.4%, whereas in groups with zinc levels >100 μg/dL at diagnosis, the mortality was only 14.8%.[14] Various studies showed evidence that low serum zinc levels can predict a poor outcome in sepsis.[13,14] The reason is related to the clinical manifestation of zinc deficiency, which is characterized by decreased immune cell function and increased incidence of infection. These manifestations were attributed to changes in immune system function, such as decreased function of B and T cells, decreased phagocytosis, and decreased production of cytokines.[13]

Compared to studies using zinc as a biomarker and predictor factor, more studies have been done to assess the role of zinc in sepsis through the effects of zinc supplementation. The role of serum zinc in neonates with EONS is also evidenced by the effectiveness of zinc supplementation. Zinc supplementation can increase the production of cytokines by helper T lymphocytes which promote cellular proliferation and differentiation. Zinc also increases the production of tumor necrosis factor-alpha (TNF-α) by monocytes, which in turn promotes phagocytotic capability.[15] Several studies have shown a significant role of zinc supplementation in reducing the mortality rate of EONS patients. A meta-analysis by Zhijun et al. showed that zinc supplementation of 10 mg/day in neonates reduced mortality (RR 0.48; 95% CI 0.25 to 0.94) by significantly increasing serum zinc levels (mean difference 81.97 μg/dL; 95% CI 34.57 to 129.37; P=0.0007) after administration for two years.[15]

A caveat of this study is that we could not fully control for other factors that could affect deterioration of sepsis, namely, the presence of other types of micronutrients that were not examined such as iron (Fe), copper (Cu), vitamin A, and other micronutrients. Further study with broader risk factor analysis is needed to evaluate the possible independence of association of serum zinc levels with EONS prognosis. In conclusion, serum zinc level is associated with the prognosis of early onset neonatal sepsis, where serum zinc levels <75 μg/dL are signficantly predictive of a worse prognosis than serum zinc levels >75 μg/dL.

Conflict of Interest

None declared.

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Original Article

Subclinical hypothyroidism in pediatric nephrotic syndrome: the correlations with albumin, globulin, and proteinuria

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Abstract

Background Nephrotic syndrome causes loss of medium-sized plasma proteins and binding proteins, resulting in thyroid hormone deficiency.

Objective To assess for potential correlations between subclinical hypothyroidism in pediatric nephrotic syndrome with albumin, globulin, and proteinuria.

Methods This cross-sectional study was conducted in the Department of Pediatrics, Hasan Sadikin General Hospital, Bandung, West Java. All types of nephrotic syndrome patients aged 1 month to < 18 years were included. Blood and urine specimens were collected from the patients for albumin, globulin, thyroid function (T3, fT4 and TSH), and proteinuria tests and analyzed with standard techniques.

Results There were 26 subjects, 20 males and 6 females. Ten subjects developed subclinical hypothyroidism, with mean albumin and thyroid-stimulating hormone (TSH) levels of 0.92 g/dL and 6.9 mIU/L, respectively. There was a negative correlation between albumin level and subclinical hypothyroidism (rpb=-0.702; P<0.001) and a positive correlation between proteinuria and subclinical hypothyroidism (r=0.573; P=0.003). Univariate logistic regression analysis revealed that globulin had no impact on the presence of subclinical hypothyroidism, but albumin and proteinuria did have such an impact. The odds ratios of albumin and proteinuria with subclinical hypothyroidism were 27.00 (95%CI 1.69 to 17.7) and 19.80 (95%CI 1.94 to 201.63), respectively.

Conclusion Subclinical hypothyroidism correlates with serum albumin level and proteinuria in nephrotic syndrome patients. The low serum albumin level has a high likelihood of subclinical hypothyroidism. **[Paediatr Indones. 2020;60:91-6; doi: http://dx.doi.org/10.14238/pi60.2.2020.91-6]**.

Keywords: *nephrotic syndrome; subclinical hypothyroidism; serum albumin; proteinuria*

Nephrotic syndrome can affect children of any age, from infancy to adolescence, but is most commonly seen among school-aged children and adolescents. Nephrotic syndrome (NS) is a glomerular disease characterized by excessive urinary protein excretion, hypoproteinemia, and edema with or without hyperlipidemia. Proteinuria occurs due to increased permeability of the glomerular capillary walls, which disrupts reabsorption in the proximal tubular epithelial cells.[1-3] Based on the response to corticosteroids, NS patients are categorized as having either steroid-sensitive nephrotic syndrome (SSNS) or steroid-resistant nephrotic syndrome (SRNS). Further definitions in SSNS include remission, relapse, frequently relapsing NS and steroid-dependent NS.[4] Children with SRNS also have a protracted clinical course and may have long-standing proteinuria.[5,6]

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Patients with NS tend to experience a state of hypoproteinemia and proteinuria. Thyroid hormone in the circulation is bound to proteins, mainly thyroid-binding globulin (TBG), prealbumin, and albumin. The proteins serve to maintain thyroid hormone levels in physiological conditions.[3,7,8] Loss of thyroid hormones may lead to low free thyroid hormone levels unless production is increased under the influence of thyroid-stimulating hormone (TSH). Furthermore, loss of albumin and TBG may reduce the binding capacity for thyroid hormones, resulting in a decrease in total triiodothyronine (T3) and thyroxin (T4) concentrations.[2,9] Nephrotic syndrome patients have a variable thyroid hormone profile. During nephrosis, thyroid hormone concentrations decrease while serum TSH concentrations increase in untreated children. Thyroid hormone levels return to normal during remission. These hormonal changes are strongly related to the degree of proteinuria and albumin levels in NS patients.[6,9] Most thyroid hormone deficiencies that occur in NS cases are subclinical.[9,10] Subclinical hypothyroidism is characterized by increased TSH levels based on references, accompanied by normal levels of fT4. Subclinical hypothyroidism can be categorized as mild or severe for TSH level increase to between 4.5-10 mIU/L or > 10 mIU. [6,11,12]

To our knowledge, no comprehensive study has evaluated thyroid function in pediatric NS cases of in our hospital. Therefore, we aimed to analyze for possible correlations between the occurrence of subclinical hypothyroidism with serum albumin, globulin, and proteinuria in NS patients in Hasan Sadikin General Hospital.

Methods

This cross-sectional study was conducted in Hasan Sadikin General Hospital, Bandung, West Java, from March to May 2019, after approval from the Hasan Sadikin General Hospital Ethics Committee. The inclusion criteria were patients aged 1 month to < 18 years, diagnosed with NS, hospitalized at Hasan Sadikin General Hospital, Bandung, and whose parents provided informed consent for participation. Patients who had thyroid abnormalities before being diagnosed with NS, liver disorders, or severe malnutrition were excluded. Patient's age, gender, type of nephrotic syndrome, and treatment was evaluated. Patients were examined about levels of thyroid function (T3, T4 and TSH levels), urea, serum creatinine, albumin, globulin, total protein, and levels of proteinuria during hospitalization by the medical personnel. Estimated glomerular filtration ratio (eGFR) was calculated using the Schwartz fomula that used serum creatinine, height and an empirical constant. The main outcomes assessed in this study were levels of T3, fT4, and TSH, in order to analyse for possible correlations between the occurrence of subclinical hypothyroidism to albumin and globulin levels, and proteinuria in NS patients.

Hypoalbuminemia was defined as serum albumin < 2.5 g/dL. Massive proteinuria was defined as urinary protein ≥ 2+ by dipstick test.[13]

Steroid-resistant NS was defined as no remission after full dose treatment of 2 mg/kg/day of prednisone for 4 weeks. Another study defined SRNS as no occurrence of remission after administration of 8 weeks of prednisone at 60 mg/body surface area/day or 2 mg/kg body weight/day for 4 weeks, followed by 40 mg/body surface area/day or 1.5 mg/kg body weight in 4 weeks.[4,13] In this study, SRNS was defined as no remission after full dose treatment of 2 mg/kg/day of prednisone for 4 weeks.

Steroid-sensitive NS included remission, relapse, frequently relapsing NS and steroid-dependent NS. Recurrent NS was defined as relapse ≥ 2 times in the first 6 months after the initial response or ≥ 4 times in a 1 year period. Remission was defined as negative or trace proteinuria (proteinuria < 4 mg/body surface area/hour) for 3 consecutive days in 1 week. Relapse was defined as proteinuria ≥ 2+ (proteinuria > 40 mg/body surface area/hour) for 3 consecutive days in 1 week. Relapse was divided into frequent and infrequent relapse. Infrequent relapse was relapse less than 2 times in the first 6 months after the initial response or less than 4 times per year, whereas frequent relapse occured more than 2 times in the first 6 months after the initial response or ≥ 4 times in 1 year period.[4,13]

Subclinical hypothyroidism (SH) was defined as an increase TSH serum levels > 4.5 mIU/L with a normal serum of fT4.[6,11,12]

Patient data collected were age, sex, anthropometric assessment of height/age and weight/age, type of corticosteroid, type of NS, type of chemo-

therapy, and kidney function assessment of glomerular filtration rate. Univariate analysis was done to describe the characteristics of the study subjects listed above. Categorical data were presented in numbers and percentages and numerical data were presented in median, minimum, and maximum values.

Bivariate analysis was done to determine potential correlations between the occurrence of subclinical hypothyroidism with serum albumin, globulin, and proteinuria using biserial point correlation, because the data types were numerical and categorical. Logistic regression analysis was done to assess the risk of serum albumin, globulin, and proteinuria on the presence of subclinical hypothyroidism. Data were tested descriptively and data analysis was carried out using *Statistical Product and Service Solution (SPSS) for Windows version 18.0,* with 95% confidence level and P value ≤ 0.05.

Results

The study was conducted on 26 children with NS. Subjects' characteristics are shown in **Table 1**.

Types of chemotherapy and corticosteroids administered are shown in **Table 2**. No significant difference was found between the occurrence of subclinical hypothyroidism and type of chemotherapy, corticosteroids, or the number of chemotherapy cycles.

The serum and urinary protein and kidney function profiles are shown in **Table 3**. The lowest total protein, albumin, and globulin levels were 2.9 g/dL, 0.2 g/dL, and 2.6 d/dL, respectively. Statistically significant differences were found between the occurrence of subclinical hypothyroidism and total protein, albumin, and proteinuria. Lower total protein, lower albumin, and higher proteinuria were associated with the occurrence of subclinical hypothyroidism (P<0.05 for all).

Thyroid function measurements are shown in **Table 4**. The median T3, fT4, and TSH levels were 0.69 ng/mL, 1.2 ng/mL, and 6.9 mIU/L, respectively, in subjects with subclinical hypothyroidism.

The correlations between serum albumin, globulin, proteinuria and subclinical hypothyroidism are shown in **Table 5**. Subclinical hypothyroidism was negatively correlated to albumin level (r=–0.702; P<0.001), indicating that subjects with low albumin levels had a high likelihood of subclinical hypothyroidism. However, globulin level had no significant correlation to subclinical hypothyroidism. In addition, a positive correlation was found between proteinuria and subclinical hypothyroidism (r=0.573; P=0.003).

Univariate logistic regression analysis was performed to determine the impact of serum albumin, globulin, and proteinuria on the presence

Table 1. Baseline characteristics of subjects

Characteristics	Subclinical hypothyroidism	
	Yes (n=10)	No (n=16)
Mean age (SD), years	4.1 (3.5)	8.0 (4.0)
Gender, n		
Male	7	13
Female	3	3
Type of NS, n		
SSNS	3	2
SRNS	7	14

SSNS=steroid-sensitive NS, SRSN=steroid-resistant NS

Table 2. Types of chemotherapy and corticosteroids

Variables	Subclinical hypothyroidism		P value
	Yes (n=10)	No (n=16)	
Mean chemotherapy cycles (SD)	3 (2)	4 (2)	0.271[a]
Type of chemotherapy, n (%)			0.091[b]
None	4	2	
Cyclophosphamide	5	14	
Mycophenolate mofetil	1	0	
Type of corticosteroid, n (%)			0.625[c]
Methylprednisolone	8	14	
Prednisone	2	2	

Note: analysis using: (a) unpaired T-test, (b) Chi-square, (c) Fisher's exact

Table 3. Serum and urinary protein and kidney function profiles

Variables	Subclinical hypothyroidism		P value
	Yes (n=10)	No (n=16)	
Median total protein (range), g/dL	4.2 (2.9-5.5)	6.0 (3.8-7.9)	<0.001[a*]
Median albumin (range),g/dL	0.92 (0.20-2.60)	2.71 (0.70-3.80)	<0.001[a*]
Median globulin (range), g/dL	3.23 (2.6-3.6)	3.27 (2.6-4.5)	0.814[a]
Median urea (range), mg/dL	25.2 (8.0-120.7)	19.2 (5.0-95.0)	0.268[b]
Median creatinin (range), mg/dL	0.27 (0.03-1.06)	0.35 (0.12-2.21)	0.510[b]
Median eGFR (range), mL/min/1.73 m^2	157 (42-771)	142 (29-327)	0.752[b]
Proteinuria, n (%)			0.012[c*]
Negative	1	11	
≥2+	9	5	

Note: analysis using: (a) unpaired T-test, (b) Mann-Whitney, (c) Chi-square

Table 4. Thyroid function

Variables	Subclinical hypothyroidism		P value
	Yes (n=10)	No (n=16)	
Median T3, ng/mL	0.69 (0.4-1.1)	1.14 (0.4-1.7)	0.002[a*]
Median fT4 (range), ng/mL	1.2 (0.2-2.2)	1.4 (0.9-2.0)	0.281[a]
Median TSH (range), mIU/L	6.9 (5.3-19.5)	1.8 (0.6-4.0)	< 0.001[b*]

Note: analysis using: (a) unpaired T-test, (b) Mann-Whitney, (c) Chi-square

of subclinical hypothyroidism in NS patients, (**Table 6**). Albumin and proteinuria had a significant impact on the presence of subclinical hypothyroidism (OR 27.00, 95%CI 1.69 to 17.7 and OR 19.80, 95%CI 1.94 to 201.63, respectively).

Serum globulin did not show statistically significant effect on subclinical hypothyroidism (P=0.805). In contrast, the odds ratio (OR) of albumin for subclinical hypothyroidism was 27.00, indicating that albumin < 2.5 g/dL increases the risk of subclinical hypothyroidism by 27.00 times. The odds ratio of proteinuria for subclinical hypothyroidism was 19.80.

Table 5. Analysis of albumin, globulin and proteinuria with subclinical hypothyroidism

Variables	Subclinical hypothyroidism	
	r coefficient	P value
Albumin, g/dL	-0.702a	<0.001*
Globulin, g/dL	-0.048a	0.407
Proteinuria	0.573b	0.003*

Note: analysis using correlation: (a) biserial point, (b) phi

Table 6. Univariate logistic regression analysis of subclinical hypothyroidism with albumin, globulin, and proteinuria

Variables	Logistic regression coefficient		P value
	Odds ratio (OR)	95% CI for OR	
Albumin, g/dL	27.00	1.69 to 17.7	0.006*
Globulin, g/dL	1.30	0.17 to 10.17	0.805
Proteinuria	19.80	1.94 to 201.63	0.012*

Discussion

Nephrotic syndrome changes the concentrations of thyroid hormones, primarily due to loss of protein in serum and urine. In our study, thyroid function was evaluated in children with nephrotic syndrome. Of the 26 NS cases, there were 20 males and 6 females. Ten out of 26 subjects had subclinical hypothyroidism, mean age at presentation of 4.1 years, and were clinically euthyroid, with increased TSH (median 6.9 mIU/L).

Previous studies have reported that in NS, thyroid hormone levels decrease while serum (TSH) levels increase. Also, several studies have found a

correlation between proteinuria, serum TSH, and T4 levels.[2] We found a direct relationship between protein excretion in urine and increased in serum TSH levels. We suggest that NS patients have an increased risk of subclinical hypothyroidism. Abnormalities in thyroid function are seen in patients with proteinuria. Specifically, TSH levels were higher in patients with proteinuric renal diseases compared to controls.[9] In addition, thyroid profiles of children with untreated NS indicated massive urinary losses of T4, T3 TBG, fT4, and fT3.[14] Our study demonstrates correlation between albumin level, proteinuria and subclinical hypothyroidism, based on the logistic regression univariate analysis (OR 27.00, 95%CI 1.69 to 17.7 and OR 19.80, 95%CI 1.94 to 201.63, respectively). Proteinuria results in loss of thyroid hormones, most probably caused by loss of thyroxin-binding globulin and albumin, thus stimulating TSH production.

A previous study found that the mean serum T3 and T4 in children during nephrosis were within normal limits, however, mean TSH was higher than normal during nephrosis. They concluded that NS patients commonly have a state of mild or subclinical hypothyroidism during proteinuria, although they are clinically euthyroid.[10] Another study reported that NS patients had an increased risk of subclinical hypothyroidism, but thyroid function returned to the normal when the non-thyroid illness was resolved.[15] We also found that serum T3 and T4 levels were in the normal range and TSH was increased.

In our study, seven children with SRNS had subclinical hypothyroidism. Prolonged proteinuria in children with SRNS may exhaust the thyroid reserve and potentiate tubular injury, resulting in impaired reabsorption of low molecular weight proteins and leading to overt hypothyroidism, if left untreated. It is possible that hypothyroidism is a consequence, not only of urinary loss of thyroid hormones, but also of a failure of the thyroid gland to compensate for this loss.[6] Actually, the low level of serum thyroid hormone can inhibit the expression of glucocorticoid receptor (GR), with manifestations of steroid resistance.[15]

A meta-analysis showed that thyroid hormone replacement therapy significantly improved the remission of the NS patients.[15] The decision to treat a child with subclinical hypothyroidisme in long-term may involve a lifetime of thyroid hormone replacement and frequent monitoring of total or free T4 and TSH levels.

The limitations of this study included not measuring free T3 and total T4 in the serum thyroid hormone profiles. Nor did we measure the total amount of urinary loss of thyroid hormones. In addition, the small number of subjects was due to using the consecutive sampling method during a limited period of time.

In conclusion, subclinical hypothyroidism in NS patients has correlations to lower albumin level and higher proteinuria. The hormonal changes are related to the degree of proteinuria and to serum albumin levels. Subclinical hypothyroidism needs to be considered in NS patients as it is a potentially health-threatening and treatable complication. Early thyroid hormone administration might improve the prognosis. Prospective, observational studies are needed to determine the etiology and pathogenesis of this health-threatening, potentially treatable complication.

Conflict of interest

None declared.

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Original Article

Full outline of unresponsiveness score as a predictor of outcomes in critically ill pediatric patients

Novita Purnamasari Assa, Dyah Kanya Wati, Ida Bagus Subanada, Soetjiningsih, Made Kardana, Made Sukmawati

Abstract

Background Mortality predictions are very important for improving service quality in the pediatric intensive care unit (PICU). The full outline of unresponsiveness (FOUR) is a new coma scale and is considered capable of predicting mortality and outcome.

Objective To assess the ability of FOUR scores to predict outcomes of critically ill patients in the PICU.

Methods This prospective cohort study included children aged 1 months - 18 years who were admitted to the PICU. Subjects were assessed by FOUR, grouped into score < 9 or score >9, and followed until outcomes were obtained. Bivariate analysis to assess the risk of death was made by cross-tabulation and the strength of the association in the form of risk ratio by Chi-square test. Multivariate analysis was done by logistic regression test.

Results Of 94 subjects, 47 had FOUR scores ≤9 and 47 subjects had FOUR >9. Bivariate analysis revealed that PICU patients with FOUR score ≤9 had a higher risk of death than those with FOUR score >9 (RR 12.5; 95%CI 3.1 to 49.8; P<0.0001). Multivariate analysis revealed that FOUR score, length of stay ≤7 days, and non-surgical disease significantly increased the risk of mortality in PICU patients (by 42.8 times, 8.9 times, and 5.9 times, respectively).

Conclusion The FOUR scores have good ability to predict the outcomes of critically ill pediatric patients. A FOUR score ≤9 at the beginning of treatment is significantly associated with the outcome of mortality during treatment in the PICU. **[Paediatr Indones. 2020;60:77-82; doi: http://dx.doi.org/10.14238/pi60.2.2020.77-82]**.

Keywords: *full outline of unresponsiveness score; critically ill; prognosis outcome; pediatric intensive care unit*

Critical illness is a condition that requires support of vital organs to prevent failure that can cause death. This support can either be mechanical or pharmacological assistance. The pediatric intensive care unit (PICU) is a facility or a separate unit, which is designed for the treatment of children with medical, surgical, trauma, or other life-threatening conditions, who require intensive care, as observation is comprehensive and specialized.[1]

Mortality in PICU patients remains very high. Several factors contribute to the outcome of critically ill patients in the PICU, such as age, sex, nutritional status, mechanical ventilation support, length of stay, type of disease, intellectual disability, cerebral palsy, and major congenital abnormalities.[2] Predicting the outcomes is very important to improving service quality in the PICU. The usual PICU mortality scoring systems include pediatric logistic organ dysfunction

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(PELOD), pediatric risk of mortality (PRISM), and pediatric index of mortality (PIM). Of the many scoring systems used, each has advantages and disadvantages. PELOD II, PRISM III, and PIM3 scores have many variables in order to cover many organ systems, so they require extensive examinations.[3-6]

The full outline of unresponsiveness (FOUR) score is a new coma scale developed with consideration of the limitations of the Glasgow coma scale (GCS) useful for intensive care. In addition to evaluating consciousness, some studies noted that FOUR was able to predict the outcomes of critically ill patients in the PICU.7-9 The FOUR cut-off score for predictors of mortality is 9.[7,10]

Studies on the role of FOUR score as a mortality predictor in critically ill children in the PICU have been limited. We aimed to assess the ability of FOUR scores to predict outcomes of critically ill patients in the PICU.

Methods

This prospective cohort study included children aged 1 month - 18 years who were admitted to the PICU, Sanglah Hospital, Bali, Indonesia from February to April 2018. Patients with intellectual disability, cerebral palsy, and major congenital abnormalities were excluded. Subjects were divided into two groups, those with FOUR score ≤9 and those with FOUR score >9, and followed until an outcome was obtained. FOUR scores were checked by residents on duty, with an inter-rater reliability of 0.890. Components of the FOUR score are shown in **Table 1**.[7] Subjects were included by consecutive sampling and sample size was calculated based on unpaired categorical comparative analytics, with alpha 0.05 and power 0.8. The minimum required sample size for each group was 47 subjects.

The FOUR score is a description of a situation without a complete response in order to provide more detailed neurological abnormalities that might give a prognosis for critically ill patients. There are four components that are valued in FOUR, namely, eye, motor, brain stem, and respiration, each of which has a maximum value of 4.[7] Data are grouped according to the cut-off point from previous study, namely (1) FOUR score ≤9 and (2) FOUR score >9.[8] Outcome is the final condition of the patient, either died or survived.

Table 1. The Full Outline of Unresponsiveness (FOUR) score[7]

FOUR score
Eye response
4 = eyelids open or opened, tracking, or blinking to command
3 = eyelids open, but not tracking
2 = eyelids closed but open to loud voice
1 = eyelids closed but open to pain
0 = eyelids remain closed to pain
Motor response
4 = thumbs up, fist, or peace sign
3 = localizing to pain
2 = flexion response to pain
1 = extension response to pain
0 = no response to pain or generalized myoclonus status
Brainstem reflex
4 = pupillary and corneal reflexes present
3 = one pupil wide and fixed
2 = pupillary or corneal reflexes absent
1 = pupillary and corneal reflexes absent
0 = absent pupillary, corneal, and cough reflex
Respiration
4 = regular breathing patterns, not intubated
3 = Cheyne-Stokes breathing pattern, not intubated
2 = irregular breathing patterns, not intubated
1 = breathing at speeds above ventilator, intubated
0 = apnea or breathing with ventilator speed.

Subject characteristics are presented descriptively. Bivariate analysis to assess the risk of death was made by cross-tabulation and risk ratio using Chi-square test. Multivariate analysis by logistic regression test was done to assess the pure effect of FOUR score in predicting mortality, with a $P<0.05$ level of significance. The data were analyzed with *SPSS 20.0* software. This study was approved by the Research Ethics Committee of Udayana Medical Faculty/ Sanglah Hospital, Denpasar.

Results

During the study period, 95 children met the age criterion, but 1 patient was excluded because of cerebral palsy. Hence, the total sample size was 94 subjects. Characteristic data collected were age, gender, nutritional status, use of mechanical ventilation, length of stay, and type of disease. Characteristics of subjects according to FOUR score group are shown in **Table 2**. Most subjects were aged ≤5 years. The majority of subjects in both groups were male and had malnutrition. Mechanical ventilation was used

by 44 subjects (93.6%) in the FOUR <9 group, and 5 (10.6%) in the FOUR >9 group. The length of stay for most subjects was ≤7 days and the most common illness types were surgery and respiratory.

The association between FOUR score and mortality in critically ill patients in the PICU was analyzed using Chi-square test. **Table 3** shows that FOUR ≤9 had a significantly higher risk of mortality than FOUR score >9 (RR 12.5; 95%CI 3.1 to 49.8; P<0.0001). Multivariate logistic regression analysis was used to adjust the confounding variables such as length of stay ≤7 days and non-surgical disease.

Table 4 shows the multivariate analysis where FOUR score, length of stay ≤7 days, and non-surgical disease significantly increased the risk of mortality in PICU patients (by 42.8 times, 8.9 times, and 5.9 times, respectively).

Discussion

The mortality of pediatric patients in the PICU is remains high. The pediatric intensive care unit of Sanglah General Hospital managed 604 critically ill patients during the period of February to April 2018. The net death rate (NDR) at the Sanglah Hospital PICU in 2016 was 19.66, while the gross death rate (GDR) was 24.08%. Several factors contribute to the outcome of critically ill PICU patients, such as age, sex, nutritional status, mechanical ventilation support, length of stay, intellectual disability, cerebral palsy, and major congenital abnormalities.[2]

Table 2. Characteristics of subjects based on FOUR score groups

Variables	Total (N=94)	FOUR score group	
		Score ≤9 (n=47)	Score >9 (n=47)
Age, n (%)			
1-11 months	28 (29.8)	17 (36.2)	11 (23.4)
1-5 years	37 (39.4)	15 (31.9)	22 (46.8)
6-10 years	11 (11.7)	4 (8.5)	7 (14.9)
11-12 years	5 (5.3)	3 (6.4)	2 (4.3)
13-18 years	13 (13.8)	8 (17.0)	5 (10.6)
Sex, n (%)			
Male	55 (58.5)	29 (61.7)	26 (55.3)
Female	39 (41.5)	18 (38.3)	21 (44.7)
Nutritional status, n (%)			
Super-obese	2 (2.1)	1 (2.1)	1 (2.1)
Obese	4 (4.3)	1 (2.1)	3 (6.4)
Overweight	4 (4.3)	4 (8.5)	0
Well nourished	35 (37.2)	18 (38.3)	17 (36.2)
Moderate malnutrition	46 (48.9)	22 (46.8)	24 (51.1)
Severe malnutrition	3 (3.2)	1 (2.1)	2 (4.3)
Mechanical ventilation support, n (%)			
Yes	49 (52.1)	44 (93.6)	5 (10.6)
No	45 (47.9)	3 (6.4)	42 (89.4)
Length of stay, n (%)			
≤7 days	64 (68.1)	28 (59.6)	36 (76.6)
>7 days	30 (31.9)	19 (40.4)	11 (23.4)
Type of disease, n (%)			
Respiratory	28 (29.8)	15 (31.9)	13 (2.7)
Cardiovascular	14 (14.9)	6 (12.8)	8 (17.0)
Neurological	12 (12.8)	8 (17.0)	4 (8.5)
Hematologic & oncologic	2 (2.1)	1 (2.1)	1 (2.1)
Endocrine and metabolic	1 (1.1)	0	1 (1.1)
Gastrointestinal	2 (2.1)	1 (2.1)	1 (2.1)
Surgery	30 (31.9)	11 (23.4)	19 (40.4)
Kidney and urinary tract	3 (3.2)	3 (6.4)	0
Tropical infection	2 (2.1)	2 (4.3)	0

Table 3. Bivariate analysis of mortality outcome and FOUR score

Variables	Outcomes		RR	95% CI	P value
	Died	Survived			
FOUR score, n (%)					
≤9	25 (53.2)	22 (46.8)	12.5	3.1 to 49.8	<0.0001
>9	2 (4.3)	45 (95.7)			

Table 4. Multivariate analysis of FOUR score as a predictor of mortality in critically ill PICU patients

Variables	Exp (B)	95% CI	P value
FOUR score ≤9	42.8	8.0 to 227.5	<0.0001
Length of stay ≤7 days	8.9	2.1 to 37.2	0.003
Non-surgical disease	5.9	1.0 to 34.0	0.048

Age is an important predictive factor on PICU patient outcomes. The younger the age, the lower the maturity of the immune system. Less mature immune systems have lower ability to eradicate pathogens, increasing the risk of death in young children. In our study, 65 subjects (69%) were aged ≤5 years. This finding was similar to other PICU study. Children less than 5 years of age had a 0.6 times higher risk of death compared to groups over 5 years.[12]

The majority of our subjects were males (55 subjects; 58.5%), similar to a Dr. Cipto Mangunkusumo Hospital study in which 66.7% of subjects were male.[13] In addition, moderate malnutrition was most common in both groups, with 22 subjects (46.8%) in the FOUR ≤9 group and 24 subjects (51.1%) in the FOUR > 9 group. Similarly, a previous study reported that 54.5% of children treated in intensive care units had moderate malnutrition.[14] Malnutrition, either directly or indirectly, is associated with high mortality. The immune systems of malnourished children are weak, so these children are susceptible to infectious diseases, especially in developing countries.[15] One study reported mortality rates of critically ill patients accompanied by malnutrition to be 2.6 times higher than in those with good nutrition.[16]

Mechanical ventilation, while providing a positive, life-saving impact, can also have a negative impact in the form of intra-pulmonary and extra-pulmonary complications.[17,18] In our study, 49 subjects (52.1%) used mechanical ventilation. Most of these were in the FOUR ≤9 group.

Length of stay is also an important predictive factor of outcome of critically ill PICU patients. Length of stay also increases mortality (30%) compared to shorter length of treatment (20%).[19] In our study, the length of stay for most subjects was ≤7 days. In the FOUR ≤9 group, 59.6% had length of stay ≤7 days and 40.4% had length of stay > 7 days. This may have been due to age, comorbidity, hypermetabolism, organ failure, and/or nutritional deficiencies.[20]

The diagnosis at PICU admission is important for determining the prognosis. A previous study in a tertiary hospital in Jakarta found that the most common diagnosis in PICU patients was CNS infection (36.7%), followed by non-CNS malignancy (20%), non-CNS infection (16.7%), and CNS malignancy (13.3%).[10] In our study, patient diagnoses were grouped according to the type of disease with surgery in 31.9%, followed by respiratory in 29.8%, cardiovascular in 14.9%, and neurological in 12.8%. This observation may have been because our hospital receives referrals from the eastern part of Indonesia, so our PICU treats various surgical cases, namely, cases of pediatric surgery, neurosurgery, trauma surgery, and thoracic-cardiovascular surgery.

The FOUR score consists of four components: assessment of brain stem reflexes, eye assessment, broad spectrum motor response, and the presence of abnormal breathing patterns as well as respiratory effort, with a rating scale of 0-4 for each component. The eye response describes the function of nerve nuclei III, IV, and VI in the mesencephalon, pons, and by two higher centers in the frontal and parieto-occipital lobes. Motor response describes the location of lesions in the brain. Examination of the brain stem reflex can help in a more complete and accurate coma depth assessment. Respiratory examination describes the normal interactions between the brain stem and cerebral cortex. The total FOUR score showed good prognostic value for predicting outcomes.[7,21]

Bivariate analysis revealed that FOUR score ≤9 had a significantly higher risk of mortality than FOUR score >9 in critically ill PICU patients (RR=12.5; 95%CI 3.1 to 49.8; P<0.0001). The association of

FOUR score with mortality based on existing references can be influenced by age, sex, nutritional status, mechanical ventilation support, length of stay, and type of disease. Multivariate analysis also showed surgical disease to have significantly lower mortality rates than non-surgical diseases. Data were grouped into surgical and non-surgical because a dichotomous nominal variable was needed to analyze for a relationship between the type of disease and outcome. Mortality was 6.7% in surgical cases and 39.1% in non-surgical cases. This difference was clinically significant. Because the difference was >15%, we subjected it to multivariate analysis because it might be a confounding factor. Non-surgical diseases included the respiratory, cardiovascular, neurological, hematologic and oncologic, endocrine, metabolic, and gastrointestinal systems, as well as kidney and urinary tract, and other disorders in accordance with the indications of PICU admission.

Multivariate analysis was performed to control for confounding variables (length of stay ≤7 days and non-surgical cases). The FOUR score ≤9 had a 42.8 times higher risk of poor prognosis in critically ill PICU patients (95%CI 8.0 to 227.5; P<0.0001). Other factors that played roles in this association between FOUR score and mortality were length of stay ≤7 days (P=0.003) and non-surgical cases (P=0.048). Factors that influence the length of stay are age, comorbidity, hypermetabolism, organ failure, and nutritional deficiencies.[20]

The limitation of this study was that the FOUR score cut-off was not self-determined, but from the previous literature.[8] In conclusion, FOUR score has good ability to predict outcomes of critically ill patients in the PICU. A FOUR score ≤9 at the beginning of treatment was significantly associated with mortality.

Conflict of interest

None declared.

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Original Article

Efficacy of high-dose methylprednisolone and cyclophosphamide in childhood-onset systemic lupus erythematosus

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Abstract

Background Systemic lupus erythematosus (SLE) is a chronic, multisystem, autoimmune disease. Untreated SLE often become progressive and lead to increased risk of mortality. Corticosteroid and cyclophosphamide remain the treatment of choice for severe SLE. Disease activity assessed with SLE Daily Activity Index (SLEDAI).

Objective To compare the disease activity of childhood-onset severe SLE at the time of diagnosis, after completion of high dose methylprednisolone, and after three month of cyclophosphamide by using SLEDAI.

Methods This study was conducted in the Division of Pediatric Allergy and Immunology, Department of Child Health, Udayana University/Sanglah Hospital, Denpasar, Bali. Subjects were SLE patient aged 0-18 years who had severe clinical manifestations. Subject received therapy combination of high dose methylprednisolone and cyclophosphamide every 2 weeks for six doses. SLEDAI score was assessed at the time of diagnosis, after completion of high dose methylprednisolone, and after three month of cyclophosphamide.

Results During the study period, 51 children were diagnosed as SLE. Twenty-one subjects were included for analysis. Median SLEDAI score at the time of diagnosis was 23 (range 13-39). SLEDAI score after three months of cyclophosphamide was decreased to 2 (range 0-14). Post hoc analysis with Wilcoxon signed-rank test showed the improvement of SLEDAI score at the time of diagnosis and after three months of cyclophosphamide was statistically significant ($Z=-4.016$, $P<0.0001$).

Conclusion SLEDAI score reduces after completion of high-dose methylprednisolone and three month of cyclophosphamide therapy. **[Paediatr Indones. 2020;60:117-24; doi: http://dx.doi.org/10.14238/pi60.3.2020.117-24]**.

Keywords: *SLE; childhood-onset; SLEDAI; high-dose methylprednisolone; cyclophosphamide*

Systemic lupus erythematosus (SLE) is a chronic, multisystem, autoimmune disease characterized by periods of increased disease activity caused by inflammation of blood vessels and connective tissue.[1] The disease can lead to various morbidities. Clinical presentations in childhood-onset SLE are largely heterogeneous and course of disease is unpredictable. Childhood-onset SLE tend to had more severe clinical manifestation compared to adult form.[1-3] The renal and central nervous system were the organs involved and had a larger portion in the childhood-onset.[2,3] Untreated

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SLE becomes progressive and increase risk of mortality. Approximately twenty percent of SLE cases develop during the first two decades of life.1,2,4 The prevalence of childhood-onset SLE was 6.3-19.3 per 100,000 in Asia. The mean age at diagnosis was 8.6–13.5 years.[4] From 2005 through 2013, 91 children with SLE were hospitalized in Dr. Cipto Mangunkusumo Hospital, Jakarta.[5]

Corticosteroids and cyclophosphamide remain the treatment of choice for severe SLE (SLE with significant renal/central nervous system/cardiac involvement).[6] The use of corticosteroids and immunosuppressive therapy was recommended by *European League Against Rheumatism* (EULAR) guidelines.[6,7] Long-term monitoring was recommended to evaluate the disease activity and adverse effect therapy, with 3-6 month assessment for mild disease and more frequent in patients with severe active disease.[7-10]

Disease activity cannot be determined by a single clinical sign or laboratory. Different measures have been developed to assess SLE disease activity including the systemic lupus activity measure (SLAM), SLE disease activity index (SLEDAI), the European consensus lupus activity measurement (ECLAM), and the British isles lupus assessment group (BILAG).[11] Overall, these disease activity measurements are accurate and reliable. SLEDAI score system easy to use, even for beginner. SLEDAI can be completed in about two minutes. Every variable is clearly defined to minimize misperception. SLEDAI score also sensitive to assess changes in disease activity. The final SLEDAI score would be from 0 (no disease activity) and 105 (the most severe disease activity). An increased in SLEDAI > 3 were considered as increased disease activity.[12]

This study aimed to compare the disease activity of childhood-onset severe SLE at the time of diagnosis, after completion of high dose methylprednisolone, and after 3 months of cyclophosphamide by using SLEDAI. Our hypothesis is there was an improvement of SLEDAI after high dose methylprednisolone and after three months of cyclophosphamide.

Methods

This study was conducted in the Division of Pediatric Allergy and Immunology, Department of Child Health, Medical School, Udayana University/Sanglah Hospital Denpasar, Bali. Data were obtained from patients medical records. We included children with severe SLE (SLE with severe clinical manifestations) based on *American College of Rheumatology/Systemic Lupus International Collaborating Clinics* (ACR/SLICC) *2012 Criteria*,[13] aged 0-18 years, from July 2015 to December 2018. Exclusion criteria were incomplete medical records and subjects who did not finish induction therapy according to protocol for severe SLE (high-dose methylprednisolone and 6 intravenous cyclophosphamide in 2-week interval).

Consecutive random sampling was performed until the minimum subject fulfilled. The minimum required number of subjects was calculated with standard deviation 2.2,[14] type I error 5%, power 80%, and mean difference 2. Data collected from the patients' medical records included age, date of diagnosis, sex, nutritional status, antinuclear antibody immunofluorescence (ANA IF), anti-dsDNA antibody, complement 3 (C3), and disease activity. Disease activity was assessed based on SLEDAI score. SLEDAI was calculated at the time of diagnosis, after high-dose methylprednisolone and after completion of intravenous cyclophosphamide. Age was defined as the chronological age stated in years. For those aged > 6 months, age was rounded up; for those aged ≤ 6 months, age was rounded down. Nutritional status was determined based on weight-for-height. Nutritional status was classified according to the *Center for Disease Control and Prevention* (CDC) 2000[15] and Waterlow criteria (weight/ideal body weight)15 as: (1) obesity > 120%, (2) overweight 111-120%, (3) well-nourish 90-110%, (4) moderate malnutrition 70-89%, (5) severe malnutrition < 70%. Short stature was defined as height-for-age below -2 standard deviation. The data of ANA IF, anti-dsDNA antibody, and C3 was obtained from subjects' medical records based on Prodia laboratory examination. ANA IF was negative if the titer ≤ 1:100 and positive if the titer > 1:100. Anti-dsDNA was determined quantitatively. Complement 3 was not checked routinely in all SLE patients due to financial issues.

The SLEDAI score was evaluated using *SLEDAI 2000/SLEDAI 2K Sheet*, which consists of 24 parameters (clinical and laboratory). The minimum SLEDAI score is 0, the maximum score is 105. An

increase in SLEDAI > 3 were considered as increased disease activity.[12] Severe SLE defined as SLE with organ involvement that may lead to irreversible damage in the affected organ, such as lung, cardiac, renal, central nervous system (CNS), and hematology involvement.[6,16] Therapy was started once severe SLE was diagnosed. Induction therapy consists of intravenous methylprednisolone 30 mg/kg/day (maximum 1000 mg) for three consecutive days, followed by cyclophosphamide 500 mg every 2 weeks for six doses (3 months).

Statistical analysis was performed using *SPSS version 20.0* (*SPSS INC*., Chicago). Mean SLEDAI at the time of diagnosis, after high dose methylprednisolone, and after three month of cyclophosphamide were compared by using Friedman test. A P value of <0.05 was considered statistically significant. This analysis was continued by Wilcoxon post-hoc test. The study protocol had been approved by the Research Ethics Commission of Universityas Udayana Medical School, Denpasar, Bali.

Results

During the period of July 2015 until December 2018, there were 51 SLE patients, 39 patients were diagnosed as severe SLE, but only 21 patients were included in the study. Eighteen patients were excluded (ten patients died before completing the induction therapy, five patients continued therapy in another

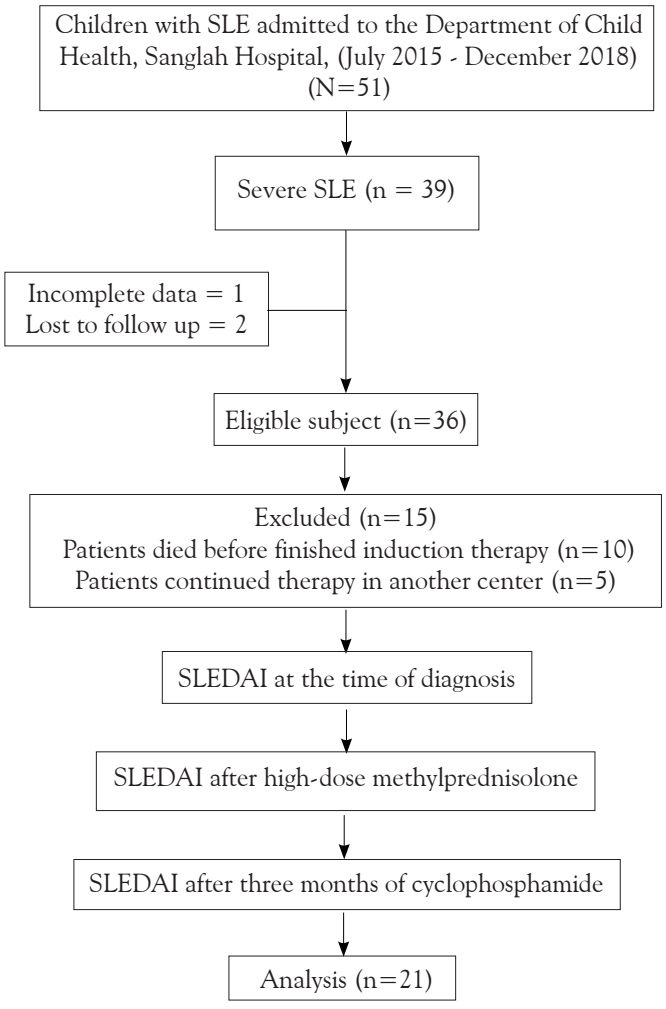

Figure 1. Study flow chart

center, one patient with incomplete medical record, two patients lost to follow up). The study flow chart is shown in **Figure 1**.

Characteristics of subject is shown in **Table 1**. The mean age at diagnosis was twelve year, the youngest subject was eight years old and the oldest subject was seventeen years old. Eighteen (85.7%) subjects were female and three (14.2%) subjects were male, the ratio of female and male was 18:3. Eight (38%) patients were malnourish whereas nine (42.8%) patients were short stature.

Table 1. Subjects characteristic

Clinical characteristics	(N=21)
Mean age at diagnosis (SD), years)	12.47 (2.47)
Age group, n	
5-10 year	1
10-18 year	20
Sex, n	
Male	3
Female	18
Nutritional status, n	
Obesity	1
Overweight	3
Well-nourish	9
Moderate malnutrition	8
Height per age, n	
Normal	12
Short stature	9
ANA IF, n	
Positive	21
Median anti-dsDNA (range), IU/mL	790 (10 – 3200)

The most clinical manifestations observed according to SLICC criteria was renal manifestation (85.7%), followed by non-scarring alopecia (76.1%), serositis (71.4%), and acute cutaneous lupus (61.9%). Neurological manifestation was found in 23% of subjects. **Table 2** shows organ involvement during clinical course of severe SLE.

Positive ANA test was found in all subject. An increased of anti-dsDNA were found in eighteen subject (85.7%). Hemolytic anemia observed in four cases (19%). Leukopenia and thrombocytopenia were found in only two subjects. Smith antibodies (anti-Sm) were found in four subjects. From twenty-one subjects, seventeen subjects underwent C3 examination. The mean C3 level at the time of diagnosis was 37.4 mg/dL.

Table 2. Major organ involvement

Organ	n
Central nervous system	5
Cardiovascular	5
Lung	5
Renal	17

According to **Table 3**, the median SLEDAI score at the time of diagnosis was 23 (13-39) and decreased to 2 (0-14) after three months of cyclophosphamide. There was a significant difference in SLEDAI score ($P<0.0001$) between subjects' group (**Table 4**).

Table 3. SLEDAI over three periods of observation

SLEDAI score	Median (range)
Total SLEDAI score at the time of diagnosis	23 (13-39)
Total SLEDAI score after high-dose methylprednisolone	13 (0-21)
Total SLEDAI score after three month of cyclophosphamide	2 (0-14)

Table 4. Analysis of total SLEDAI of each group

SLEDAI	Mean rank	P value*
Total SLEDAI at the time of diagnosis	3.00	<0.0001
Total SLEDAI after high-dose methylprednisolone	1.98	
Total SLEDAI after three month of cyclophosphamide	1.02	

*Friedman test

To determine when differences actually occured in the clinical course, we run the post-hoc Wilcoxon analysis on the different combinations of the related groups (**Table 5**). There were statistically significant improvement of total SLEDAI score between the time of diagnosis and after high-dose methylprednisolone, the time of diagnosis and after three months of cyclophosphamide, and between after high-dose

Table 5. Relationship between observed groups

SLEDAI	Score change(Z)	P value*
Total SLEDAI at the time of diagnosis – after high-dose methylprednisolone	-4.018**	<0.0001
Total SLEDAI at the time of diagnosis – after 3 months of cyclophosphamide	-4.016**	<0.0001
Total SLEDAI after high-dose methylprednisolone - after 3 months of cyclophosphamide	-3.923**	<0.0001

*Wilcoxon signed rank test **Based on positive ranks

methylprednisolone and after three months of cyclophosphamide.

Discussion

The mean age of SLE onset in this study was twelve years, with most subjects diagnosed above ten years of age. Several studies found a similar result. A previous stud reported the mean age of disease onset was 10.5 years, with the most prevalent age > 10 years,[17] while another study reported the mean age at diagnosis was 11.9 years.[2] The two studies mentioned above reported that female: male ratio was 4.5:1 and 5:1, respectively.[2,17]

The most clinical manifestations observed according to SLICC criteria were renal manifestation (85.7%), followed by non-scarring alopecia (76.1%), serositis (71.4%), and acute cutaneous lupus (61.9%). Neurological manifestation was found in 23% of subjects. A previous study reported the most common clinical finding was hematologic manifestations, followed by arthritis and malar rash.[2] Another study reported that the most common symptoms were rash on skin/face and arthralgia.[17] In this study, 61.9% of subjects were having acute cutaneous lupus. Mucocutaneous manifestation was reported up to 90% of pediatric SLE. Malar or "butterfly" rash is pathognomonic for SLE.[1] The common organ involved in childhood-onset SLE was the kidney. A study reported 82% of childhood-onset SLE among Vietnamese had renal manifestation.[18] Another study reported 62.8% of their subject had renal manifestation.[19] In contrast, a study in Singapore reported the renal involvement was only 40%.[2] Therefore periodic renal evaluation should be performed in all childhood-onset SLE.

Positive ANA test was found in all subjects. A previous study reported nearly all (98%) their subjects had positive test.[2] Another studies reported positive ANA test in about 91% of childhood-onset SLE had positive ANA.[17,20] Positive ANA test has a high sensitivity of 98%, but low positive predictive value (10%) and specificity as low as 36%.[21] Several disease or syndrome frequently associated with a positive ANA test are SLE, juvenile rheumatoid arthritis, juvenile dermatomyositis, mixed connective tissue disease, psoriatic arthritis, infection (Lyme), and malignancy (acute lymphocytic leukemia).[1,17,20-23] ANA was comprised as collections of autoantibodies against various nuclear proteins. The presence of high titers of ANA should arouse suspicion of autoimmune disease.[23] Positive ANA titers were categorized as low (1:40 to 1:80), medium (1:160 to 1:320), and high (≥ 1:640).[23] Up to 10% of healthy children and adolescent may present with isolated high titer of ANA.[23] Low ANA titer (< 1:640) should be ignored unless the child is systemically ill and show clinical manifestations of SLE.[21] A study found that children with high ANA titers have a higher mortality risk.[23]

In this study, anti-dsDNA were found in eighteen subject (85.7%), anti-Sm (19%), and anti-ribosomal P (9.5%). A study found 85% of childhood-onset SLE had positive anti-dsDNA.20 Anti-dsDNA, anti-Smith antibodies (anti-Sm), and anti-ribosomal P autoantibodies are pathognomonic of SLE diagnosis. Other autoantibodies that may be found at SLE are anti-RNP, anti-SSA/Ro, and anti-SSB/La.[24,25] Anti-ds DNA, found in > 75% of childhood-onset SLE and can be used as a parameter of SLE activity.1,20 Anti-dsDNA antibodies in SLE has high specificity. Anti-Sm has the highest specificity, but low sensitivity for SLE. Both anti-dsDNA and anti-Sm antibodies are associated with renal involvement.[26] In this study, from eighteen subjects who have positive anti-ds DNA, only one subject did not have renal manifestation. In contrast, a previous study found that anti-dsDNA was not associated with increased frequency of renal involvement. Two subjects have strong positive anti-ribosomal P. The organ involved in both subjects was central nervous system, with seizure as the chief complaint. One subject has strong positive anti-SSA, anti-SSB, and recombinant Ro52. The main clinical manifestation in this subject was large pericardial effusions and anemia. One subject has strong positive anti-RNP/SM and anti-Sm, where the symptom is predominantly in the central nervous system and liver.[2]

Reduced serum complements C3 and C4 have been used as a marker of active lupus disease relapse for decades.[1,25] A previous study found the median C3 and C4 level were 56 (range 9-173) mg/dL and 8 (range 2-35)mg/dL, respectively. In this study, the median C3 level when SLE confirmed was 37.4 (range 17-85) mg/dL.[2]

The aim of SLE management was to achieve

remission, reduces disease activity, and prevent toxicities from medications. The approach of management of childhood-onset SLE was achieved by using glucocorticoids and immunosuppressive drugs. In our center, severe SLE is treated with intravenous methylprednisolone 30 mg/kg to a maximum of 1 gram (for three consecutive days) then followed with glucocorticoids 2 mg/kg/day. Cyclophosphamide is primarily used as immunosuppressive drugs in severe SLE, including lupus nephritis, life-threatening organ involvement, and neuropsychiatric manifestations. There are two regimens of intravenous cyclophosphamide recommended for induction therapy in lupus nephritis. The first one is low dose cyclophosphamide (500 mg intravenous once every 2 weeks for a total of 6 doses), followed by maintenance therapy with daily oral azathioprine or daily oral mycophenolate mofetil. The second one is high dose cyclophosphamide (500-1000 mg/m^2 intravenous once a month for 6 doses), followed by mycophenolate mofetil or azathioprine.[27] The European protocol for management of lupus nephritis consist of low-dose intravenous cyclophosphamide (500 mg) every 2 weeks for six doses. This regimen was effective for the initial treatment.[28]

We evaluate SLEDAI score at the time of diagnosis, after patients had three consecutive high-dose intravenous methylprednisolone, and after six doses of low-dose intravenous cyclophosphamide. Median SLEDAI at the time of diagnosis was 23 (range 13-39), after 3 consecutive high-doses of methylprednisolone was 13 (range 0-21), and after 3 months of cyclophosphamide was 2 (range 0-14). A previous study found the mean SLEDAI at the time of diagnosis was 13.[29] The subjects with both renal and CNS manifestations show an improvement after 6 months therapy, where the mean SLEDAI decrease from 18.2 (SD 10.5) to 1.9 (SD 1.7) (P=0.0001).[29] Another study also found an improvement of SLEDAI 6 months after induction therapy where the decrease of SLEDAI was 11.07.[11] A study observed SLEDAI at the time of diagnosis and then repeated every 3 months for about 12 months of observation. They found the median SLEDAI at time of diagnosis was 16 (range 8-34), and it decreased 3 months later.[5]

The Friedman test found the difference of SLEDAI score among all three groups was statistically significant (P<0.0001). Post hoc analysis with Wilcoxon signed-rank test found there was an improvement of SLEDAI score at the time of diagnosis and after high-dose methylprednisolone Z=-4.018; P<0.0001), at the time of diagnosis and after 3 months of cyclophosphamide (Z=-4.016; P<0.0001), after high-dose of methylprednisolone and after 3 months of cyclophosphamide (Z=-3.923; P<0.0001). These analysis shows that SLEDAI after high-dose methylprednisolone and after three month of cyclophosphamide was improved significantly.

There were several limitation in this study. Some laboratory parameters need to assess according to SLEDAI such as DNA binding and complement level were not checked routinely due to clinical judgement and cost. Also, the small sample size limited the strength of the study. In conclusiom, SLEDAI scores reduce after completion of high-dose methylprednisolone and three month of cyclophosphamide therapy.

Conflict of interest

None declared

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Original Article

Influence of screen time and sleep duration on obesity in early adolescents

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Abstract

Background Behavioral and environmental factors increase the risk of obesity. Many Indonesian children have their own smartphones and engage in excessive screen time, which may negatively impact their nutritional status and sleep duration. However, to our knowledge no studies have explained the magnitude of the influence of screen time and sleep duration on obesity in early adolescents.

Objective To analyze the influence of screen time and sleep duration on obesity in children aged 10-13 years.

Methods This case-control study was done from April to June 2019 in Semarang, Central Java. Subjects comprised 70 obese and 70 non-obese children, based on CDC body mass index-for-age percentiles. Subjects were recruited from 7 primary schools. Children's screen time and sleep duration data were collected from the modified 3DPAR questionnaire. Statistical analysis was conducted using Chi-square and logistic regression tests.

Results Obesity had significant associations with short sleep duration (aOR=4.20; 95%CI 1.80 to 9.78) and long computer screen time (OR=4.13; 95%CI 1.28 to 13.25). Total screen time on other media or all media combined were not associated with obesity since both the obese and non-obese groups spent >2 hours/day on screens. Short sleep duration was the dominant risk factor for obesity (OR=4.08; 95%CI 1.78 to 9.35).

Conclusion Short sleep duration (<9 hours/day) is associated with and a dominant risk factor for obesity in children aged 10-13 years. However, screen time is not associated and not influential as a dominant risk factor for obesity, despite the high odds of obesity in children with long computer screen time (>2 hours/day). **[Paediatr Indones. 2020;60:154-9; doi: http://dx.doi.org/10.14238/pi60.3.2020.154-9]**.

Keywords: *screen time; sleep duration; obesity; early adolescents*

Obesity is a global health problem that increases the risk of morbidity and mortality. *The World Health Organization* (WHO) stated that obesity was associated with a greater number of global deaths than underweight.[1] The prevalence of children with overweight and obesity worldwide has increased sharply in recent years.[2] In 2016, the WHO reported that there were 124 million obese children and that number was expected to increase beyond the incidence of underweight in 2022, if the condition was not treated immediately and seriously.[3] Indonesia had the highest prevalence of obesity in children aged 5-12 years (18.8%), with 10.8% overweight and 8% obese.[4]

According to the Republic of Indonesia Ministry of Health, obesity occurs due to an imbalance between diet and physical activity.[5] The rapid development of technology has contributed to the increasing prevalence of obesity by leading to a sedentary or inactive lifestyle.[6] Indonesian children consume a

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variety of media at home, such as television, cell phones, video players, radios, gaming devices, and computers.[7] More than 80% of children aged 10-13 years in Indonesia already own a personal cell phone or smartphone.[8] Excessive screen time is thought to be related to obesity and decreased sleep duration.[9,10] Nonetheless, to the best of our knowledge, no other study has investigated the magnitude of the influence of screen time and sleep duration on obesity among early adolescents. Hence, we aimed to analyze the influence of screen time and sleep duration on obesity in children aged 10-13 years.

Methods

This case-control design was conducted in 70 obese children and 70 non-obese children, aged 10-13 years. Subjects were recruited from 7 primary schools in Semarang, Central Java, from April to June 2019 by consecutive sampling. The inclusion criteria were body mass index (BMI) > 95th percentile for the obese group or 5th to 95th percentile (based on CDC curve according to gender and age)[11,12] for the non-obese group, as well as parental informed consent and willingness to fill the parenting style questionnaire. Sick children were excluded.

The independent variables in this study were screen time and sleep duration, while the dependent variable was obesity. Subjects' gender and physical activity as well as parental income and parenting style were considered to be confounding variables. Physical activity were classified as low (<1 hour/day) or enough (≥ 1 hour/day) physical acitivity showed by how long subjects spent their time in a day for doing some physical aerobic exercise or muscle strengthening exercise, or bone strengthering exercise.

Subjects' weights and heights were measured using body impedance analysis (BIA) and a stadiometer, respectively. Screen time and sleep duration were obtained by the modified 3DPAR questionnaire.13 The 3DPAR is used for self-reporting daily habits by filling in activity codes in 30-minute intervals over 24-hour periods. It has been used by the University of South Carolina13 and tested with Alpha Cronbach reliability test in Semarang after translation to the Indonesian language.[13] The 3DPAR questionnaire was filled by children and parents answer the parenting style questionnaire.

To minimize recall bias, a quick interview session was done after children filled their questionnaires to help them recall their activities in the previous 3 days and to clarify or make sure data was correctly filled. Subjects were sub-classified based on long screen time (>2 hours/day) or short screen time (≤ 2 hours/day), according to the AAP recommendation for screen time in children.[14,15] Subjects were also sub-classified based on long sleep duration (≥ 9 hours/day) or short sleep duration (< 9 hours/day), according to CDC recommendation for sleep duration among school-aged children.[16]

The data were analyzed using *IBM SPSS Statistics 20*. The hypothesis that long screen time and short sleep duration influence obesity (OR≠1) in children aged 10-13 years was tested using Chi-square (for data with expected count >5), Fisher's exact test (for data with expected count <5), Mantel-Haenszel test (to assess potential confounding factors), and multiple logistic regression test (to determine which variable was a dominant risk factor). Results with P values < 0.05 were considered to be significant. The study was approved by the Ethics Committee, Universitas Diponegoro University Medical School/Dr. Kariadi Hospital. The Board of Education permitted the study to be conducted in primary schools in Semarang.

Results

Of 174 students, 140 children met the inclusion criteria, 70 per group. The characteristic data collected were gender, sleep duration, and physical activity (**Table 1**).

The mean sleep duration and screen time for all media, smartphone-screen time, TV-screen time, and computer-screen time for obese, non-obese, and all subjects are shown in **Table 2**. The mean time of sleep duration, and screen time varied in each media. Note that mean screen times in the obese group were higher than screen times for all subjects.

The cross-tabulation of screen time, sleep duration, and confounding variables with obesity were analyzed by Chi-square test (**Table 3**). Gender, sleep duration, and computer screen time were significantly different between the obese and non-obese groups.

Table 1. Characteristics of the study subjects

Characteristics	n (%)
Gender	
Male	59 (42.1)
Female	81 (57.9)
Sleep duration	
Short	90 (64.3)
Long	50 (35.7)
Screen time for all media	
Long	121 (86.4)
Short	19 (13.6)
Screen time-TV	
Long	68 (48.6)
Short	72 (51.4)
Screen time-smartphone	
Long	61 (43.6)
Short	79 (56.4)
Screen time-computer	
Long	18 (12.9)
Short	122 (87.1)
Physical activity	
Short	104 (74.3)
Long	36 (25.7)

There was more obese boys compared to girls, more obese adolescents who had shorter sleep duration and longer computer screen time.

Mantel-Haenszel test was done to control for the confounding variable of gender (**Table 4**). After controlling for confounders, short sleep duration retained its significant association with obesity (P=0.001). However, long computer screen time was no longer significantly associated with obesity.

Logistic regression test was done for variables with P values <0.25, in order to assess for a dominant risk factor (**Table 5**). Male gender and short sleep duration were both significant risk factors for obesity. Through multivariate analysis, obtained an equation to count the probabilities (P) for being obese in boys with short sleep duration was 83.1%.

Discussion

In our study, significantly more obese children had short sleep duration (77.1%) than non-obese children (51.4%). The adjusted odds ratio after controlling for confounding variables was >1 (aOR 4.20; 95%CI 1.81 to 9.78; P=0.001).

Screen time was not associated with obesity, since both groups had mean screen time >2 hours/day, which exceeded the American Academic of Pediatrics (AAP) recommended limit. The mean screen times for all subjects were as follows: all media 4.73 hours/day (OR 1.13; 95%CI 0.43 to 2.98; P=0.805), smartphone 2.6 hours/day (OR 1.50; 95%CI 0.77 to 2.95; P=0.233), and TV 2.3 hours/day (OR 1.000; 95%CI=0.52 to 1.94; P=1.000). However, there was a significant relationship between computer screen time and obesity (P=0.012) before controlling for confounding variables (Mantel Haenszel test P>0.05), with a crude odds ratio of >1 (crude OR 4.13; 95%CI 1.28 to 13.25). Short computer screen time was observed in 94.3% of the non-obese group and 80% of the obese group. Mean computer screen time for all subjects was <2 hours/day.

Multivariate analysis revealed that short sleep duration and male gender were significantly associated with obesity, with odds ratios >1 [(OR 4.1; 95%CI 1.78 to 9.35; P=0.001) and (OR 6.4; 95%CI 2.89 to 14.35; P<0.001), respectively]. After adjusting for male gender, short sleep duration (<9 hours/day) remained a significant risk factor for obesity (aOR 4.20). This result was in agreement with a Yogyakarta and Bantul Regency study which showed a significant relationship between shorter sleep duration and obesity.[17]

A number of possible pathways linking sleep deprivation with obesity have been suggested. Chronic sleep deprivation may be related to hormonal distur-

Table 2. Subjects' mean sleep duration and screen times by group

Variables	Obese (n=70)	Non-obese (n=70)	All subjects (N=140)
Mean sleep duration (SD), hours	8.03 (1.56)	8.7 (1.33)	8.37 (1.48)
Mean screen time (SD), hours	5.47 (3.18)	3.98 (1.63)	4.73 (2.63)
Mean TV-screen time (SD), hours	2.34 (1.81)	2.32 (1.56)	2.33 (1.69)
Mean computer-screen time (SD), hours	0.96 (1.79)	0.46 (0.93)	0.72 (1.44)
Mean smartphone-screen time (SD), hours	3.22 (3.03)	1.98 (1.38)	2.6 (2.43)

Table 3. Comparison of sleep duration, screen time, and sociodemographic variables between obese and non-obese adolescents

Variables	Group		OR (95% CI)	P value
	Obese n (%)	Non-obese n (%)		
Gender				
Male	43 (61.4)	16 (22.9)	5.38 (2.57 to 11.23)	<0.001+
Female	27 (38.6)	54 (77.1)		
Sleep duration				
Short	54 (77.1)	36 (51.4)	3.18 (1.54 to 6.61)	0.001+
Long	16 (22.9)	34 (48.6)		
Screen time				
Long	61 (87.1)	60 (85.7)	1.13 (0.43 to 2.98)	0.805+
Short	9 (12.9)	10 (14.3)		
Screen time-TV				
Long	34 (48.6)	34 (48.6)	1.00 (0.52 to 1.94)	1.000+
Short	36 (51.4)	36 (51.4)		
Screen time-smartphone				
Long	34 (48.6)	27 (38.6)	1.50 (0.77 to 2.95)	0.233+
Short	36 (51.4)	43 (61.4)		
Screen time-computer				
Long	14 (20)	4 (5.7)	4.13 (1.28 to 13.25)	0.012+
Short	56 (80)	66 (94.3)		
Physical activity duration				
Short	52 (74.3)	52 (74.3)	1.00 (0.469 to 2.134)	1.000+
Long	18 (25.7)	18 (25.7)		

+Chi-square test

bances, such as decreased leptin release by adipocytes or low leptin level, and increased ghrelin level.[18] Ghrelin acts as a potent stimulator of appetite, while leptin acts as a suppressor.[19] Low leptin has a much stronger effect than high leptin levels, which have been associated with leptin resistance usually observed in obesity.[20,21] Sleep deprivation also plays a role in reduced inhibition of orexigenic activity in the hypothalamus (orexin as a strong appetite stimulant that rises in response to low leptin level).[22,23] These neurohormonal changes lead to increased hunger or appetite and desire for calorie-dense food that may result in more weight gain in the short term and obesity in the long term.[24-26] Another possible pathway is that reduced sleep duration may cause fatigue, leading to reduced physical activity.[27] Chronic low physical activity and uncontrolled appetite or food intake causes an imbalance between energy consumption and energy expenditure that may lead to obesity.[5]

Table 4. Association between obesity and sleep duration and computer-screen time, adjusted for gender

	aOR (95% CI)[a]	P value
Sleep duration (short)	4.20 (1.81 to 9.78)	0.001[α]
Computer-screen time (long)	3.64 (0.95 to13.91)	0.059

[α]Mantel-Haenszel test

Table 5. Multivariate analysis of sleep duration, gender, as well as computer and smartphone screen times

Variables	B	P	OR	95%CI
Sleep duration (short)	1.40	0.001*	4.08	1.78 to 9.35
Gender (male)	1.86	<0.001*	6.44	2.89 to 14.35
Computer screen time (long)	0.89	0.174	2.44	0.67 to 8.84
Smartphone screen time (long)	-0.18	0.653	0.83	0.37 to 1.87
Constant	-1.681	<0.001	0.19	

*significance <0.05

We found that screen time (all media, smartphone-media only, or TV-media only) was not influential as a risk factor for obesity in children, but screen time for all media categories were longer in the obese group than in the non-obese group. Similarly, a Denpasar, Indonesia study reported that obese children had longer screen time (and greater than average screen time among all groups) than non-obese children.[9]

The odds of obesity in children with long computer screen time (>2 hours/day) were 4.1 times higher than in children with short computer screen time (≤2 hours/day), but gender was a confounder in this relationship. Furthermore, the mean computer screen time in all subjects was less than 2 hours/day. These findings differed from smartphone and TV screen time, perhaps because children use TV more than computers.[28] A number of possible mechanisms linking long screen time to obesity have been suggested, including sleep deprivation. Short sleep duration has been associated with obesity.[29] Another possible mechanism is that screen time displaces the time spent doing physical activity. However, physical activity duration was not associated with obesity in our study. Moreover, a study showed that a reduction in screen time only gave a slight increase in physical activity, and screen time is not the only factor for developing obesity.[30]

We found that short sleep duration was influential as a dominant risk factor for obesity by 4.1 times. Male gender, a confounding variable, was also influential as a dominant risk factor for obesity. The odds of obesity in 10 to 13-year-old boys were 6.4 times higher than in girls of similar age range. If a boy also had a short sleep duration, his probability of obesity was 83.1%. Boys tend to have higher risk of obesity because they have higher average energy and high carbohydrate-dense intake.[31] Leptin is also thought to play a role at this age, and its level varies by gender.[32]

The limitations of this study were that data were taken from self-reported questionnaires and not measured by an objective instrument to measure sleep duration and screen time. Recall bias and overestimates may have influenced our findings.

Our study provides evidence that significantly more obese children have short sleep duration than non-obese children. In addition, short sleep duration is a dominant risk factor for obesity in children aged 10-13 years. Mean screen times for smartphone, TV, and all media in both groups are each longer than 2 hours/day, and these three types of screen time are not associated with obesity. In addition, computer screen time is not influential as a dominant risk factor for obesity, although the odds of obesity in children with long computer screen time is quite high.

Conflict of Interest

None declared.

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Original Article

Validation of the Indonesian Version of *Modified Checklist for Autism in Toddlers*: a diagnostic study

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Abstract

Background Autism is a developmental disorder for which early detection in toddlers is recommended because of its increased prevalence. *The Modified Checklist for Autism in Toddlers* (M-CHAT) is an easy-to-interprete tool that can be filled out by parents. It has been translated into the Indonesian language but needs to be validated.

Objective To evaluate the diagnostic validity of the Indonesian version of M-CHAT in detection of autism spectrum disorder in Indonesia.

Methods A diagnostic study was conducted at Sanglah Hospital, Denpasar, Bali, from March 2011 to August 2013. Pediatric outpatients aged 18 to 48 months were included. The Indonesian version of the M-CHAT tool was filled by parents. Autism assessment was done according to the *Diagnostic and Statistical Manual of Mental Disorders,* 4th edition (DSM-IV-TR). The assessment results were analyzed with the MedCalc program software, in several steps: (i) reliability of M-CHAT; (ii) description, distribution, and proportion to determine the characteristics of the subjects of research; and (iii) validity of M-CHAT compared to the gold standard DSM-IV-TR by a receiver operating characteristic curve and several area under the curve cut-off points, in order to assess the sensitivity, specificity, positive predictive value, negative predictive value, and positive and negative likelihood ratio, accompanied by the 95% confidence interval of each value.

Results The Indonesian version of M-CHAT in toddlers had 82.35% sensitivity and 89.68% specificity, using the cut-off point of more than 6 failed questions.

Conclusion The Indonesian version M-CHAT translated by Soetjiningsih has optimal diagnostic validity for detection of autism in toddlers. **[Paediatr Indones. 2020;60:160-6; doi: http://dx.doi.org/10.14238/pi60.3.2020.160-6]**.

Keywords: *M-CHAT; autism; screening; validity*

Autism is a severe disorder of development, affecting children below 36 months of age. Autism often goes undetected in toddlers, especially in urban children with low socio-economic status.[1,2] To our knowledge, there have been no studies reporting autism prevalence in Indonesia. A Bandung (West Java) study reported that 20–30% of children below five years of age have symptoms of delayed development.[3] According to a 2009 report from the Indonesian Autism Foundation, there were more than 102 therapeutic centers and 13 schools for autism in Indonesia.[4]

From 2006, the *American Academy of Pediatrics* (AAP) has recommended using the algorithm for developmental surveillance and screening for early identification of autism spectrum disorder (ASD), because it allows early intervention, etiologic investigation, and counseling regarding the risk of recurrence.[5] Of the ASD screening tools, the *Checklist for Autism in Toddlers*

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(CHAT) was designed specifically as a level 1 screening tool. It is available at no cost to practitioners for use in primary care and only requires 5 minutes to complete the questionnaire. Because of its low sensitivity, Robins modified the CHAT to the *Modified Checklist for Autism in Toddlers* (M-CHAT) to better identify children at risk for any ASD.[5] The M-CHAT is more cost- and time-efficient than other tools. It only requires about 5-10 minutes for parents to complete the survey, with 23 yes/no items that are easy to understand. The M-CHAT can be used to screen children aged 18 to 48 months. In the United States, community- and clinical-based studies on M-CHAT reported 85% of sensitivity and 93-100% of specificity.[1,5-7]

The M-CHAT was translated into the Indonesian language by Soetjiningsih, with permission from the author. This Indonesian version of M-CHAT has been widely used in Indonesia, but M-CHAT use and validity has not been reported. Hence, this study was conducted to evaluate the diagnostic validity of the Indonesian version of M-CHAT for detection of ASD in Indonesia.

Methods

This cross-sectional study was conducted in the Child Growth and Development Clinic of Sanglah Hospital, Denpasar, Bali, from March 2011 to August 2013. Children aged 18 to 48 months whose parents brought them to the clinic and agreed to participate were included in this study. We excluded children with global inability in language expression or verbal communication (because of hearing loss or palatoschisis), and those with global motor deficits or cognitive delays such that they could not pass the cognitive/developmental assessment (for example, cerebral palsy, hydrocephalus, or other severe congenital deformities).

Estimation of sample size was calculated by a single proportion sample method, with P=80%, α=0.05, Zα=1.96, d=30%, and the 4.8% proportion of children with ASD aged 18 to 48 months who came to the Child Growth and Development Clinic of Sanglah Hospital by 2009.[8] A total of 143 subjects, the minimum required sample size, were included in this study.

This study was undertaken in the following three phases: (i) translation, back translation, cultural adaptation, and a short pilot study to obtain the final Indonesian version of M-CHAT to be used in Indonesia; (ii) the reliability study; and (iii) the validity study.

The M-CHAT questionnaire was translated into the Indonesian language by Soetjiningsih with guidance that, rather than being literal, the translation should had semantic, linguistic, and cultural equivalence. The resulting version was then back-translated by a native English speaker who was bilingual, and sent to the original authors to compare it to the original M-CHAT. After several exchanges of opinion with the original author and making certain amendments, the Indonesian version of the M-CHAT questionnaire was approved by the original author. The Indonesian language M-CHAT questionnaire consists of 6 critical questions and 17 other questions, total 23 questions.

The reliability study of M-CHAT was done to evaluate the variability of the instrument and observer (parents). Our reliability study included 15 parents from the Child Growth and Development Clinic of Sanglah Hospital without inclusion nor exclusion criteria. The Indonesian version of M-CHAT was administered to the parents twice, at the first visit and at a visit 1 month later.

The reliability test for the Indonesian version of M-CHAT was done by calculating the coefficient of test-retest (intraobserver) reliability, in which 15 parents participated. This study used Bland-Altman curve as parameter of reliability test.[9]

Parents were accompanied by a resident familiar with the content of the questions in the Indonesian M-CHAT, so that they could request an explanation if needed. The authors were not directly involved in this aspect of the study in order to remain blinded to the parents' answers. Completed questionnaire was collected and Bland-Altman analysis was carried out (M-CHAT1). The first result then will be compared with the result of second analysis which was done one month later (M-CHAT2).

Children of both genders aged 18-48 months who visited our clinic with parents were screened for inclusion. Subjects' parents provided written informed consent. All subjects were evaluated by the authors using the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV-TR) criteria from the *Centers for Disease Control and Prevention*

(CDC) methodology. The ASD cases were identified and systematically defined using the DSM-IV-TR criteria from CDC. The authors were blinded to the M-CHAT results. The results of the M-CHAT and DSM-IV-TR assessments were merged by way of the research subject identity forms.

All collected data were entered and analyzed by the *MedCalc program software*, in several steps: (i) reliability of M-CHAT; (ii) description, distribution, and proportion to determine the characteristics of the study subjects; and (iii) validity of M-CHAT compared to the gold standard DSM-IV-TR by a receiver operating characteristic (ROC) curve and several area under the curve (AUC) cut-off points, in order to assess the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive and negative likelihood ratio (LR), accompanied by the 95% confidence interval of each value.

The study was approved by the Medical and Health Research Ethics Committee of Universitas Udayana Medical School/Sanglah Hospital, Denpasar.

Results

A total of 175 children were screened for this study. We excluded 32 children with global developmental delays. A Bland-Altman plot (**Figure 1**) revealed the systematic distinction between measurements and identify possible outliers. Mean difference was the estimation of bias and standard deviation from difference of random fluctuations around the mean. This study was clinically important because the difference was within the range of SD 1.96. The P value obtained from this study was <0.001 with 95%CI -0.725 to 0.725 (SD 1.31). Blant-Altman plot determined whether language in the Bahasa MCHAT questionnaire represented language that intelligible by respondens (parents). If the plot was between +1.96 and -1.96, the study could be continued. If the plot was scattered beyond the SD 1.96, the Bahasa in the MCHAT translated quiesionnaire should be improved first so it can be equally understood by all the respondens.

The characteristics of subjects are presented in **Table 1**. Of 143 study subjects, the median age was 33 months, ranging from 18-48 months. More boys participated than girls, with a ratio of 1.9:1. Subjects' nutritional status was mostly well-nourished (94.4%),

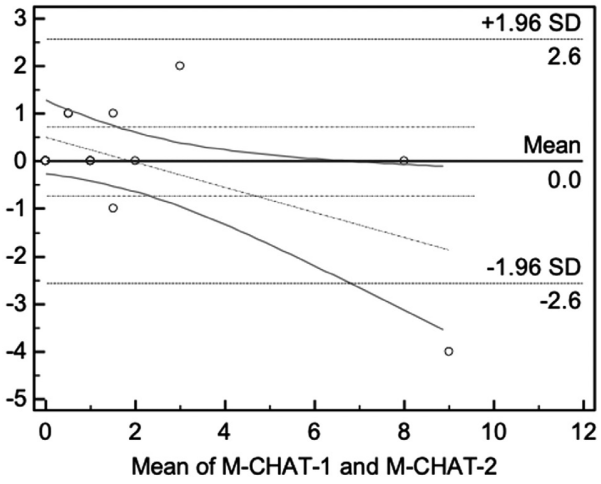

Figure 1. Bland-Altman curve

with only 5.6% malnourished. Most fathers (74.8%) did not have a college education, nor did mothers (80.4%). Only 19.6% of mothers completed university. Using the DSM-IV-TR, we identified 17 (11.9%) children with autism.

The cut-off M-CHAT score was determined by the intersection between sensitivity and specificity for the AUC and the coordinates of the ROC curve.[10,11] The AUC values evaluated were from 50%, the worst rating, up to 100%, the best value.[11] The ROC curves showed that the M-CHAT score of the study (**Figures 2** and **3**), had a good diagnostic value because the curve was far above the 50% line and closer to 100%. The AUC were 91.2% (95%CI 85.2% to 95.3%) for M-CHAT critical questions and 93.7% (95%CI 88.3% to 97.1%) for M-CHAT total questions (P<0.001).

The cut-off point was established clinically and statistically in accordance with the clinical expectations and interests,[11] by the ROC curve coordinates. The diagnostic value for the critical items of M-CHAT at various cut-off points revealed that the best validity was achieved if failure occurred on more than 1 question of the six critical questions on the Indonesian version of M-CHAT (sensitivity 82.35% and sensitivity 91.27%).

The AUC revealed that the best validity for the total M-CHAT questions was if failure occurred on 5 or more out of 23 questions (sensitivity 88.24% and specificity 85.71%). The diagnostic values for the critical and total items of the Indonesian version of

Table 1. Characteristics of subjects

Characteristics	Autism (DSM-IV-TR) (n = 17)	Total (N = 143)
Sex, n (%)		
Male	14	94 (65.7)
Female	3	49 (34.3)
Median age (range), months	38 (29 to 48)	33 (18 to 48)
Nutritional status, n (%)		
Malnourished	1	8 (5.6)
Well-nourished	15	127 (88.8)
Overweight	1	8 (5.6)
Maternal education, n (%)		
Elementary	0	13 (9.1)
Junior high	5	27 (18.9)
Senior high	10	75 (52.4)
University	2	28 (19.6)
Paternal education, n (%)		
Elementary	0	14 (9.8)
Junior high	4	26 (18.2)
Senior high	10	67 (46.8)
University	3	36 (25.2)

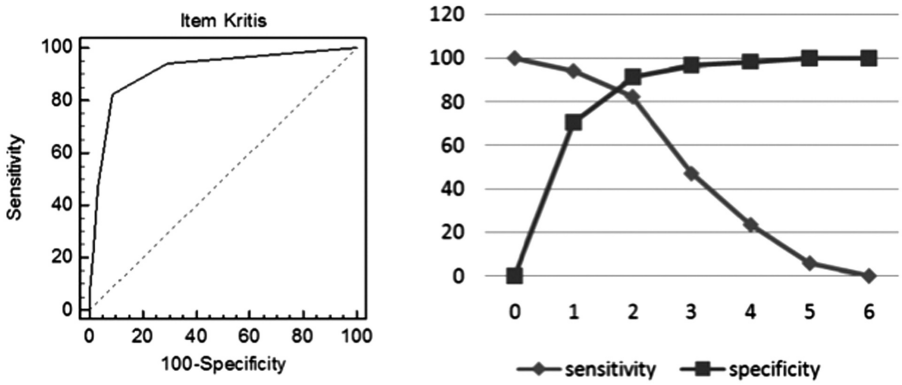

Figure 2. ROC curve and AUC cut-off for critical questions of M-CHAT

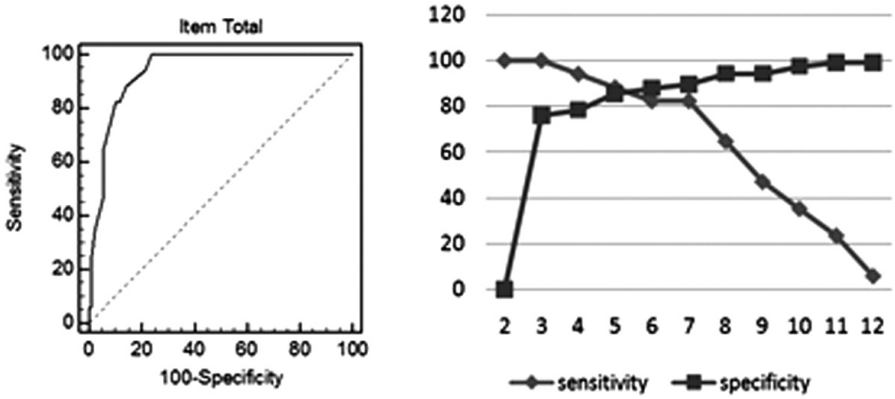

Figure 3. ROC curve and AUC cut-off for total questions of M-CHAT

M-CHAT at various cut-off points are shown in **Table 2** and **Table 3**, respectively.

The value of the likelihood ratio is varying from zero to infinity. A strong positive result of diagnostic test provides positive LR >6, while a strong negative result will gives the negative LR <0.2.10,12 Table 3 shows that at the >6 item failure cut-off point with 82.35% sensitivity and 89.68% specificity, the positive LR was 7.98 and negative LR was 0.2, which fulfilled the above criterion.

Table 2. Diagnostic values of critical M-CHAT items compared to the gold standard at various cut-off points

Cut-off	Sensitivity, % (95%CI)	Specifity , % (95%CI)	PPV, % (95%CI)	NPV, % (95%CI)	Positive LR, % (95%CI)	Negative LR, % (95%CI)
>0	94.12 (71.3 to 99.9)	70.63 (61.9 to 78.4)	30.2 (18.2 to 44.5)	98.9 (94.0 to 100)	3.21 (2.4 to 4.3)	0.083 (0.01 to 0.6)
>1	82.35 (56.6 to 96.2)	91.27 (84.9 to 95.6)	56.0 (34.9 to 75.6)	97.5 (92.7 to 99.5)	9.43 (5.1 to 17.3)	0.19 (0.07 to 0.5)
>2	47.06 (23.0 to 72.2)	96.83 (92.1 to 99.1)	66.7 (34.9 to 90.1)	93.1 (87.4 to 96.8)	14.82 (5.0 to 44.0)	0.55 (0.3 to 0.9)

Table 3. Diagnostic values of total M-CHAT items compared to the gold standard at various cut-off points

Cut-off	Sensitivity, % (95%CI)	Specifity , % (95%CI)	PPV, % (95%CI)	NPV, % (95%CI)	Positive LR, % (95%CI)	Negative LR, % (95%CI)
>3	94.12 (71.3 to 99.9)	78.57 (70.4 to 85.4)	37.2 (23.0 to 53.3)	99.0 (94.6 to 100)	4.39 (3.1 to 6.3)	0.0075 (0.01 to 0.5)
>4	88.24 (63.6 to 98.5)	85.71 (78.4 to 91.3)	45.5 (28.1 to 63.6)	98.2 (93.6 to 99.8)	6.18 (3.9 to 9.8)	0.14 (0.04 to 0.5)
>5	82.35 (56.6 to 96.2)	88.10 (81.1 to 93.2)	48.3 (29.4 to 67.5)	97.4 (92.5 to 99.5)	6.92 (4.1 to 11.7)	0.2 (0.07 to 0.6)
>6	82.35 (56.6 to 96.2)	89.68 (83.0 to 94.4)	51.9 (31.6 to 71.7)	97.4 (92.6 to 99.5)	7.98 (4.6 to 14.0)	0.2 (0.07 to 0.6)
>7	64.71 (38.3 to 85.8)	94.44 (88.9 to 97.7)	61.1 (35.7 to 82.7)	95.2 (89.8 to 98.2)	11.65 (5.2 to 25.9)	0.37 (0.2 to 0.7)

Discussion

The autism prevalence at the Child Growth and Development Clinic, Sanglah Hospital, Bali, was 9.7% for children aged 18-48 months. After excluding children with global delayed development, the prevalence rose to 11.9%. In a population-based study, a previous study reported a prevalence of 2.66%.[5] A previous study estimated an ASD prevalence of 8.5 per 1,000 children aged 3 to 5 years in the United States.[13] Research on the 14 sites in the United States revealed a prevalence of 11.3 per 1,000 children aged 8 years.[14] A community-based study in South Korea noted an ASD prevalence of 2.64% (95%CI 1.91 to 3.37) in children aged 7-12 years.[15] A study conducted two stages of community-based studies in Spain and reported prevalences of 0.92% and 0.29% of children aged 18-36 months.[16]

We found more autism in boys than in girls, with ratio of 4.7:1. Our finding was similar to that of a 2008 surveillance study in the US which reported a ratio of 5:1.[14] Eldin *et al.*[17] studied children aged 18 to 124 months in 9 Arabic countries, and reported 84.4% boys and 15.6% girls in their autism subjects (5.4:1).

The best age for autism screening in children is still debated. The AAP recommended autism screening for all children aged 18 to 24 months.[18] Firstly, M-CHAT was used to screen autism for children aged 16 to 30 months. Robins[19] included 4,797 children aged 14 to <27 months in her study. Also, a Thai study included 48 children aged 18 to 36 months.[20] In addition, another study included 18,989 children aged 16 to 30 months in their study.[18] Several longitudinal studies screened children at an early age, then reevaluated them at 2 to 4 years old. The authors concluded that M-CHAT validity was high, although the children had reached >30 months of age.[19]

The ROC curve for M-CHAT showed the

best sensitivity when failing seven or more items of M-CHAT, similar to results from previous study.[5] However, another study reported 87% sensitivity and 94% specificity for the Thai language M-CHAT with failure criteria cut-off of 8 or more questions.[20]

The earlier study found 85 to 87% of sensitivity and 93 to 99% of specificity, with 80% positive predictive value and 99% negative predictive value. Another study resulted in a 95-99% sensitivity and 95-99% specificity.[21] Aakre[22] noted that the M-CHAT has been available in Spain, Turkey, China and Japan.

The Spanish M-CHAT had good sensitivity (100%), specificity (98%), and NPV (100%), as well as moderate PPV (35%) if failure occurred on more than 1 question of critical question or more than 2 question of total question.[16] The Arabic M-CHAT had 86% sensitivity, 80% specificity, and good PPV (88%).[17] In addition, a study modified the Chinese language CHAT-23 and M-CHAT questions into a new version of 15 questions, with 100% sensitivity and 96.5% specificity if failing of 5 or 6 of 15 questions.[23] We used the Indonesian version of M-CHAT translated by Soetjiningsih and found 82.35% sensitivity (95%CI 56.6 to 96.2%) and 89.68% specificity (95%CI 83.0 to 94.4%) if failing more than 6 of 23 questions (**Table 3**).

The likelihood ratio is a semi-quantitative measurement of assessing a diagnostic test that reveals how big a diagnostic procedure will change the possibility of a disease that is calculated from sensitivity and specificity of a diagnostic test.[12] This ratio expresses the magnitude of the likelihood that ill patients would have a certain diagnostic test result divided by the possibility of well patients having the same test results.[10] The positive LR >6 and the negative LR <0.2 in a study indicates that the Indonesian M-CHAT is a good instrument because it can change the hypothesis against the probability of disease of pre-test to post-test.[12] Our LRs for a cut-off of failing >6 items were 7.98 (95%CI 4.6 to 14.0) for positive LR and 0.2 (95%CI 0.07 to 0.6) for negative LR.

Pre-test probability is the likelihood of a person having a disease prior to diagnostic test, also defined as the prevalence of the disease based on clinical and demographic characteristics.[10] The prevalence of our study was 9.7%. After excluding children with global developmental delay, the prevalence was 11.9%.

Screening instruments need high sensitivity to exclude a disease diagnosis for normal results. For conditions with a low prevalence, a test with high specificity is more important than one with high sensitivity.[10] Using a cut-off of >6 items of the total M-CHAT items, specificity was good at 89.68%. The specificity was higher than sensitivity (**Table 3**), indicating that the Indonesian version of M-CHAT is a suitable screening tool for autism.

The pre-test odds of this study were calculated from probability divided with (1-probability), resulting 0.135. The post-test odds is the multiplication value of pretest odds with positive LR, i.e. 1.078. Thus, the post-test probability according to Bayes theory could be calculated, i.e., the post-test odds divided with (1+post-test odds), amounting to 51.87%.

The limitations of our study was that we did not perform CHAT evaluation on the subjects which suspected with the M-CHAT, as some other studies did, we directly performing DSM-IV-TR on the first visit. In addition, this study was not community based, so the community-based autism prevalence remains unknown. This research needs to be continued with a larger sample size for a higher level of precision and narrower validity.

In conclusion, the Indonesian version of M-CHAT translated by Soetjiningsih is a suitable screening tool for autism. It has optimal diagnostic validity in detection of autism for failure criteria of more than 6 of 23 questions, with 82.35% sensitivity and 89.68% specificity. Of 6 critical M-CHAT questions, a failure of two or more had 82.35% sensitivity and 91.27% specificity. As such, the tool can be applied to children aged 18 to 48 months whose parents fill the form directly. If the result of the screening reveals suspected autism, parents are advised to seek expert opinion.

Conflict of Interest

None declared.

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Original Article

Impact of albumin levels on clinical outcomes in children underwent abdominal surgery

Rismala Dewi, Freddy Guntur Mangapul Silitonga, Irawan Mangunatmadja

Abstract

Background Patients underwent abdominal surgery and had hypoalbuminemia were at risk of post-operative complications. The prognostic role of albumin levels in children with abdominal surgery remains unclear.

Objective To investigate the impact of albumin levels on clinical outcomes related to the complications in children with abdominal surgery.

Methods This was a retrospective cohort study on children aged 29 days to 18 years, who underwent abdominal surgery, had serum albumin levels measured at pre-operative and within 48 hours post-operatively, and hospitalized in Paediatric Intensive Care Unit of Dr. Cipto Mangunkusumo Hospital, Indonesia. The primary outcomes were post-operative complications (sepsis, surgical site infection, shock), length of stay in PICU, dehiscence, relaparotomy, and postoperative mortality.

Results This study recruited a total of 201 children. Pre- and post-operative serum albumin levels of ≤ 3.00 g/dL were found in 15.4% and 51.2%, respectively. Pre- and post-operative serum albumin levels of ≤ 3.00 g/dL were associated with higher risk of post-operative sepsis (RR 3.4; 95%CI 1.54 to 7.51) and relaparotomy (RR 3.84; 95%CI 1.28 to 1.49). The median of length of PICU stay was 4 days longer in children with pre-operative serum albumin levels ≤ 3.00 g/dL (P<0.001).

Conclusion Hypoalbuminemia condition in children undergo abdominal surgery is associated with increased risk of post-operative sepsis, longer length of stay in PICU, and risk of relaparotomy. **[Paediatr Indones. 2020;60:149-53; doi: http://dx.doi.org/10.14238/pi60.3.2020.149-53]**.

Keywords: *hypoalbuminemia; clinical outcomes; abdominal surgery; pre- and postoperative*

Systemic changes can be resulted from major surgery as body response to trauma.[1] Previous studies proved that compared to the pre--operative state, post-operative albumin level was significantly reduced.[2] Surgery causes the increment of capillary permeability which leads to albumin and plasma leakage, resulting in hypoalbuminemia.[3] During laparotomy, 6-24% from total mass of circulating plasma protein is leaked to peritoneal cavity.[4] Serum albumin level can be used to identify post-operative patients with high -risk of morbidity and mortality.[5,6] A study using multiple regression model found that first day of post-operative albumin level was independently associated with post-operative complications.[6]

Many studies found that albumin levels less than 3 g/dL were associated with post-operative complications.[7,8] Albumin level less than 3.5 g/dL was associated with respiratory complications (pneumonia in 30 days post-operative, delayed ventilator weaning,

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and reintubation), surgical site infection, dehiscence, longer hospitalization period, and increasing one-year mortality risk.[9-11]

Patients underwent abdominal surgery and had hypoalbuminemia were at risk of post-operative complications and higher risk of mortality.[8-11] However, the prognostic role of albumin level in children with abdominal surgery remains unclear. Therefore, we aim to investigate the impact of albumin levels on clinical outcomes related to the complications in children with abdominal surgery.

Methods

This was a retrospective cohort study conducted at Dr. Cipto Mangunkusumo Hospital, Jakarta, on children aged 29 days to 18 years who admitted in Paediatric Intensive Care Unit Dr. Cipto Mangunkusumo Hospital, between January 2015 and December 2017. Data were obtained from medical records. Inclusion criteria were subjects underwent laparotomy abdominal surgery and had serum albumin levels measured pre-operatively and within 48 hours post-operatively. Subjects with incomplete data were excluded. Independent variable was < 48 hours post-operative albumin level while the dependent variables included post-operative complications (sepsis, surgical site infection, shock), length of stay in PICU, dehiscence, relaparotomy, and post-operative mortality. Hypoalbuminemia was defined as serum albumin levels ≤ 3.00 g/dL. Association between serum albumin levels and clinical outcomes were analysed using Chi-square method for categorical data and Mann-Whitney for numerical data. This study had been approved by Research Ethics Committee, Universitas Indonesia Medical School.

Results

During 3 year-period time, there were 231 children underwent laparotomy, but only 201 subjects were eligible. Most common indications of laparotomy were intraabdominal tumours (16.5%), followed by choledochal cysts (14.5%), adhesiolysis (14.5%), biliary atresia (14.5%), and perforated appendicitis (7.9%). Subjects' characteristics are listed in **Table 1**. Pre-operative and post-operative hypoalbuminemia were identified in 15.4% and 51.2% of subjects, respectively.

Both pre-operative and post-operative hypoalbuminemia were associated with higher risk of post-operative sepsis, but neither with surgical site infection nor dehiscence. Pre-operative hypoalbuminemia were associated with relaparotomy (**Table 2**). The median for length of stay in PICU was four days longer in subjects with pre-operative hypolbuminemia (**Table 3**).

Table 1. Demographic characteristic of study subject

Characteristics	N=201
Gender, n(%)	
Boys	93 (44.63)
Girls	108 (53.7)
Age, n(%)	
0-5 years	137 (68.2)
5-10 years	25 (12.4)
10-15 years	27 (13.4)
15-18 years	12 (6.0)
Nutritional status, n(%)	
Normal-overweight-obese	110 (54.7)
Wasted	62 (30.8)
Severely wasted	29 (14.4)
Type of surgery, n(%)	
Emergency	34 (16.9)
Elective	167 (83.1)
Pre-operative albumin level, n(%)	
≤3.00 g/dL	31 (15.4)
>3.00 g/dL	170 (84.6)
Post-operative albumin level, n(%)	
≤3.00 g/dL	103 (51.2)
>3.00 g/dL	98 (48.8)
Pre-operative albumin transfusion, n(%)	
Yes	27 (13.4)
No	0
Post-operative albumin transfusion, n(%)	
Yes	18 (8.9)
No	9 (4.5)
Surgical site infection, n(%)	
Yes	8 (3.9)
No	193 (96.1)
Dehiscence, n(%)	
Yes	8 (3.9)
No	193 (96.1)
Relaparotomy, n(%)	
Yes	16 (7.9)
No	185 (92.1)
Mortality, n(%)	
Yes	14 (6.9)
No	187 (93.1)

Table 2. Association between albumin level and clinical outcomes after laparotomy procedures.

Outcomes/parameter	Sepsis			Surgical site infection			Dehiscence			Relaparotomy		
	Yes	No	RR (95%CI)	Yes	No	RR (95%CI)	Yes	No	RR (95%CI)	Yes	No	RR (95%CI)
Pre-operative albumin level												
≤ 3.0 g/dL	19	12	3.4 (1.54 to 7.51)	2	29	1.88 (0.36 to 9.70)	1	30	0.77 (0.09 to 6.54)	6	25	3.84 (1.28 to 11.49)
> 3.0 g/dL	54	116	P=0.002	4	164	P=0.357	7	163	P=1.000	10	160	P=0.021
Pre-operative albumin level												
≤ 3.0 g/dL	48	55	2.55 (1.40 to 4.63)	7	96	7.07 (0.85 to 58.58)	6	7	2.96 (0.58 to 15.08)	11	92	2.22 (0.74 to 6.65)
> 3.0 g/dL	25	73	P=0.002	1	97	P=0.066	2	96	P=0,280	5	93	P=0.114

RR=relative risk; CI=confidence interval

Table 3. Median length of stay in PICU based on albumin levels

Outcomes	Albumin level, g/dL		P value
	≤ 3.0	> 3.0	
Median length of stay (range), days			
Pre-operative	8 (2-33)	4 (1-64)	<0.001
Post-operative	5 (1-64)	4 (1-38)	0.025

Mortality rate among subjects with pre-operative serum albumin level of ≤ 3.00 g/dL was 19.2%, while those with albumin >3.00 g/dL had mortality rate of 5.6%. A Kaplan Meier curve showed a better survival in subject with normal albumin compared to those with pre-operative hypoalbuminemia, despite a P=0.254 from Log Rank (Mantel-Cox) with α=0.05.

Discussion

This study found that children with pre- and post-operative serum albumin level of ≤ 3.00 g/dL had higher risk of post-operative sepsis compared to those with normal serum albumin levels. This result was similar with a previous study that found albumin level was inversely proportional to post-operative sepsis.[12] In this study, we were not able to show any associations between pre- and post-operative serum albumin levels with surgical site infection and dehiscence. This results were not accordance with the results of a previous study that found 24% of post-laparotomy patients who had dehiscence were hypoalbuminemia.[13] Hypoproteinaemia contributed in inflammation phase elongation, fibroplasia disruption, collagen proliferation and synthesis, neo-angiogenesis, and wound remodelling. Other factors, i.e. anemia, nutritional status, presence of other diseases, degree of wound contamination, surgery urgency, surgical site infection, non-proper surgery preparation, and surgery timing, also affected the presence of dehiscence.[13,14] In 8 subjects in this study who had dehiscence, we identified the contributing factors were malnutrition, burst abdomen, and haemophilia with inhibitors that disrupted wound healing phase since coagulation until remodelling phase.

Pre-operative serum albumin level of ≤3.00 g/dL was associated to higher risk of relaparotomy. A previous study showed that hypoalbuminemia was a risk factor for relaparotomy. From all patients underwent laparotomy, 54% were found hypoalbuminemia with mean albumin level of 3.4 g/dL. As much as 71% of all patients underwent relaparotomy due to anastomosis dehiscence and 66% from patients underwent relaparotomy due to burst abdomen were found to have hypoalbuminemia.[15]

In this study, the lower the albumin level (either pre- or post-operatively), the longer hospitalization period was found. This results were in accordance with study by Hennessey *et al.*[16] that showed hypoalbuminemia was associated significantly with longer hospitalization period compared to those with normal serum albumin levels (19.5 days *vs.* 12 days). Another study found that patients with albumin level between 3.0 and 3.4 g/dL had 2 (95% CI 1.83 to 2.34) days longer median post-operative hospitalization period compared to those with albumin level >3.4 g/dL.[17] A study in adults in Thailand showed that patients with non-hypoalbuminemic had significantly shorter length of hospital stay [6.8 (SD

2.6) days] compared to those with albumin level of < 3.5 g/dL [(9.6 (SD 4.7) days].[18]

The lower albumin level also associated with higher mortality rate. Subjects with post-operative serum albumin level ≤ 3.00 g/dL had mortality rate of 19.2%. The cause of mortality in all subjects was septic shock. A study found mortality rate of 40%, with mortality risk of 9%, 29%, 67%, and 83% in patients with hyperalbuminemia (>4.5 g/dL), normal serum albumin levels (3.5-4.5g/dL), moderate hypoalbuminemia (2.5-3.5 g/dL), and severe hypoalbuminemia (<2.5 g/dL), respectively.[19]

No significant difference was found in mortality rate among post-operative serum albumin levels of ≤3.00 g/dL compared to those with albumin of >3.00 g/dL. This was explained by three subjects with malignancies and one subject with hemophilia in the albumin > 3.00 g/dL subjects group, which showed that albumin level was not the only factors affecting mortality in these subjcects. Mortality in this study was caused by pre-operative septic shock that aggravating malignancies existed. Hemophilia with inhibitor also affected coagulation phase as the initial process of wound healing. Blood losses were also predicted to play a role in mortality.[20]

Limitations of this study included the bias that could be happened due to incomplete data in medical records. Serum albumin levels sometimes were not measured because of patient's condition, i.e. emergency situation for surgery. This condition made subjects were excluded from the study. Protocol for albumin transfusion also differed during the study period due to the National Health System regulation regarding to albumin transfusion.

In conclusion, hypoalbuminemia is associated with increased risk of post-operative sepsis, longer length of stay in PICU. , and risk of relaparotomy.

Conflict of Interest

None declared.

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Original Article

Reduced levels of circulating natural killer cells in children with celiac disease

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Abstract

Background Celiac disease (CD) is an autoimmune disease characterized by malabsorption. Serologic testing for CD consists of Ig A type of antitissue transglutaminase (tTG), antiendomysium (EMA). These tests are helpful in monitoring adherence to the gluten-free diet (GFD). Natural killer (NK) cell count alterations have been reported in various diseases, such as cancer, Crohn's disease, malnutrition, and autoimmune disorders.

Objective To compare peripheral blood NK cell counts in children with celiac disease (CD) to healthy controls. The second aim was to analyze for possible correlations between NK cells (CD3-/CD16+, CD56+) and tissue transglutaminase (tTG)- IgA and tTG-IgG, as well as endomysial antibody EMA-IgA indicating . gluten sensitivity.

Methods Fifty children with CD were compared to 48 healthy children as controls, with similar age and sex distribution. Peripheral blood NK cell counts were measured by flow cytometry.

Results The median (P25-P75) ages of the 50 celiac patients (23 male; 46%) and 48 controls (21 male; 44%) were 10 (2-17) years and 9 (3-17) years, respectively. Mean follow-up duration was 3 years, ranging from 1-10 years. All CD patients had positive tTG-IgA and EMA-IgA tests. while it was negative in all (100 %) control patients. The absolute number of circulating CD16+ NK cells (259.52 *vs.* 1404.36 μ/L) and CD56+ NK cells (366.24 *vs.* 2440.46 μ/L) were significantly lower in the celiac group than the control group ($P<0.05$ for both). The absolute numbers of circulating white blood cells (7785 *vs.* 8165 μ/L) and lymphocytes (3106 *vs.* 3173 μ/L) were not significantly different between the celiac and control groups ($P>0.05$ for both). Correlation analysis between the absolute number of circulating NK cells and tTG-IgA, tTG-IgG, and EMA-IgA levels in CD patients revealed no significant relationships ($P>0.05$ for all).

Conclusions Peripheral blood NK cell count are significantly lower in celiac patients than controls, hence, decreased NK cell counts may be an abnormal feature seen in autoimmune diseases. NK cell count in celiac patients has no significant correlations to tTG-IgA, tTG-IgG, or EMA-IgA levels. Therefore, NK cell count may be inappropriate marker for monitoring compliance to a gluten free diet. **[Paediatr Indones. 2020;60:125-30 ; doi: http://dx.doi.org/10.14238/pi60.3.2020.125-30]**.

Keywords: *celiac disease; natural killer cells*

Celiac disease (CD), also known as gluten-sensitive enteropathy, is a T cell-dependent chronic inflammatory disease of the proximal small intestine caused by permanent intolerance to gluten.[1] In Western countries, the prevalence of CD in childhood has been estimated to range from 1:80 to 1:300.[2] Even though CD etiology is still not completely understood, both environmental and genetic factors are believed to be involved in the pathogenesis of CD. When gluten-containing substances such as wheat, barley,

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and rye are ingested in HLADQ2 and/or HLA-DQ8 positive individuals, T cells are activated to produce cytokines, which destroys both adjacent enterocytes and the structure of the lamina propria by secreting matrix metalloproteinases. Ultimately, increased intraepithelial lymphocytes (IELs) in small intestinal mucosa lead to malabsorption.[3,4]

Natural killer (NK) cells make up approximately 15% of peripheral lymphocytes and play crucial roles in both innate and adaptive immune responses. They develop in the bone marrow and can migrate to various tissues, such as liver, lung, gastrointestinal tract, and peripheral blood. NK cells are categorized into two subsets according to their surface markers CD16 and CD56, with CD56 as the major subset of NK cells.[5] Similar to "helper" T cells, NK cells in the gastrointestinal tract are important for protecting against infectious pathogens and preserving the intestinal epithelium.[6] The activity and number of circulating NK cells may vary in malignancy, malnutrition, autoimmune disease, as well as digestive disorders such as Crohn's disease and CD.[7]

The aim of our study was to investigate the number of peripheral blood NK cells (CD3-/CD16+, CD56+) in children with and without CD and to investigate for potential correlations between NK cells and tissue transglutaminase (tTG) IgA and IgG, as well as endomysial antibody (EMA)-IgA. To our knowledge, this is the first study in children with CD to simultaneously evaluate both the absolute number of circulating NK cells and their possible correlations to tTG-IgA, tTG-IgG, and EMA-IgA.

Methods

This study was carried out at the Department of Pediatric Gastroenterology of Van Education and Training Hospital in Van, Turkey, between January and June 2018. Fifty children with classic CD aged 2-17 years and 48 healthy children (similar age and sex distribution) were included in the study. The diagnosis of CD was done according to the *European Society for Pediatric Gastroenterology, Hepatology, and Nutrition* (ESPGHAN) criteria.[8] Biopsies of the small intestine were performed for all patients with positive serum tTG-IgA and EMA-IgA tests, and evaluated according to the modified *Marsh Classification*.[9] All celiac subjects had enteropathy of Type III-c, according to Marsh's criteria. Celiac patients with comorbid diseases such as diabetes mellitus, IgA deficiency, or Down syndrome were excluded from the study. The study was approved by the Ethics Committee for Non-invasive Clinical Research at Karabuk University. Subjects' parents provided written informed consent.

For NK cell count measurements, peripheral venous blood specimens were collected in heparin-containing tubes. NK cells were purified from peripheral blood mononuclear cells (PBMC) by using the Human NK Isolation Kit II (Becton, Dickinson and Company BD Biosciences, San Jose, USA). Following isolation, NK cells were phenotypically identified as CD3-/CD16+ or CD56+ cells by flow cytometry.

The data were analyzed with *SPSS version 21.0* software for Windows. Results are expressed as median (P25-P75). Shapiro-Wilk test was used to determine the normality of data distribution. Values of white blood cell count, absolute lymphocyte count, absolute CD16+ NK cell count, absolute CD56+ NK cell count, serum tTG-IgA, tTG- IgG, and EMA-IgA levels had abnormal data distribution, by Shapiro-Wilk test, therefore, median values (interquartile range) between groups were determined and compared using Mann-Whitney U test. Height and body weight values were compared with independent T-test because of normal data distribution between groups. Correlation analyses were evaluated with Spearman's correlation test. A P value of less than 0.05 was considered to be statistically significant.

Results

The median age of the 50 celiac patients (23 male; 46%) and 48 controls (21 male; 44%) were 10 (P25-P75 2-17) years and 9 (P25-P75 3-17) years, respectively. There were no statistically significant differences between the two groups with respect to age or gender (**Table 1**). Mean follow-up duration of celiac patients was 3 years, ranging from 1-10 years. All CD patients had positive tTGA and EMA-IgA tests. On the other hand, the tTG and EMA-IgA tests of the controls were negative. The absolute number of circulating CD16+ NK cells in the CD

group was significantly lower than that in the control group (259.52 *vs.* 1404.36 μ/L, respectively; P<0.05) (**Figure 1**).

Similar to circulating CD16+ NK cells count, the absolute number of circulating CD56+ NK cells in the CD group was significantly lower than that in the control group (366.24 *vs.* 2,440.46 μ/L, respectively; P<0.05) (**Figure 2**). There were no statistically significant differences between the two groups with respect to white blood cell (7,785 *vs.* 8,165 μ/L) and lymphocyte counts (3,106 *vs.* 3,173 μ/L) (P>0.05) (**Table 1**). In the CD group, correlation analyses of circulating NK cell count with tTG-IgA, tTG-IgG, and EMA-IgA levels revealed no significant relationships (P>0.05) (**Tables 2** and **Table 3**).

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

Variables	CD group (n=50)	Control group (n=48)	P value
Median age (P25-P75), years	10 (2-17)	9 (3-17)	0.674[a]
Males, n (%)	23 (46)	21(44)	0.710
Mean body height (SD), cm	128.92 (21.59)	131.56 (18.91)	0.632[b]
Mean body weight (SD), kg (SD)	28.74 (12.62)	31.83 (11.85)	0.597[b]
White blood cell (P25-P75, µ/L	7,785 (3200-22790)	8,165 (4280-17920)	0.114[a]
Lymphocyte (P25-P75, µ/L	3,106 (1140-5760)	3,173 (763-8140)	0.166[a]
CD16+ NK cells (P25-P75, µ/L	259.52 (39.65-965.5)	1,404.36 (79.87-8,505)	0.021[a]
CD56+ NK cells (P25-P75, µ/L	366.24 (111.52-1016.32)	2,440.46 (27.66-1,1025)	0.017[a]
tTG -IgA (P25-P75, RU/mL	133 (40-300)	N/A	0.001[a]
tTG -IgG (P25-P75, RU/mL	90 (24-380)	N/A	0.001[a]
EMA-IgA(P25-P75, RU/mL	120 (30-295)	N/A0	0.001[a]

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

Table 2. Correlation between CD16+ NK cell counts with tTG -IgA, tTG-IgG, and EMA-IgA levels

Parameters	r Value[a]	P value
tTG -IgA RU/mL	0.850	0.56
tTG -IgG RU/mL	-0.431	0.76
EMA IgA RU/mL	0.639	0.64

[a]Spearman's rank correlation

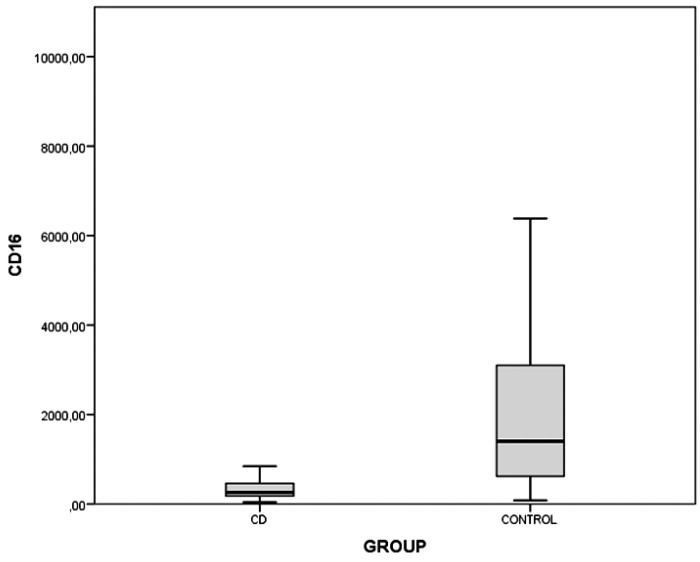

Figure 1. Comparison of median CD16+ NK cell counts in the CD and control groups

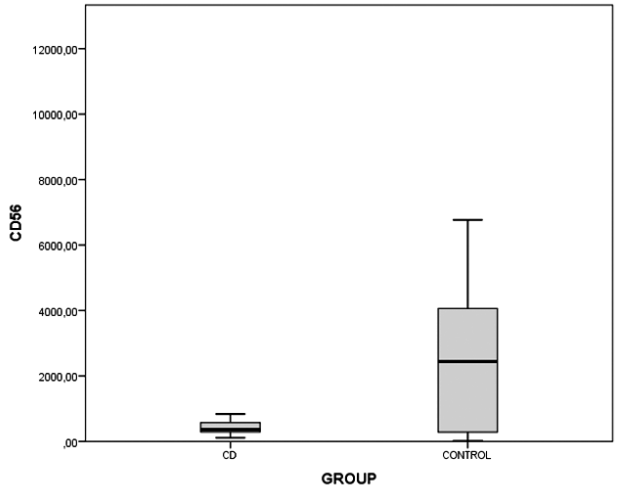

Figure 2. Comparison of median CD56+ NK cell counts in the CD and control groups

Table 3. Correlation between CD56+ NK cell count with tTG -IgA, tTG-IgG, and EMA-IgA levels

Parameters	r Value[a]	P value
tTG -IgA RU/mL	0.710	0.62
tTG -IgG RU/mL	-0.820	0.57
EMA IgA RU/mL	-0.176	0.52

[a] Spearman's rank correlation

Discussion

In our study, children with CD had lower absolute number of peripheral blood NK cells than controls. In addition, no correlations were observed between NK cell counts and tTG-IgA, tTG-IgG, or EMA-IgA levels. Few studies have evaluated blood NK cell counts in celiac children, so there were a limited number of studies to which we compared our results.

The NK cells, physical epithelial barriers, phagocytic leukocytes, as well as dendritic cells are the main components of the innate immune system. An immunoregulatory role of NK cells has been found to be either disease-controlling or promoting disease severity by producing and releasing cytokines and chemokines during activation.[10] CD16+ and CD 56+ NK cells are found predominantly in the peripheral blood.[11] In our study, NK cell counts were defined as CD3-/CD16+ or CD56+ lymphocytes. As mentioned previously, both qualitative and quantitative NK cell variations have been reported in various diseases and conditions.[12] A study reported that the number of CD3-/CD56+ NK T cells in patients with psoriasis were significantly lower than in the controls ($P<0.05$).[13] Likewise, in a study evaluating the number of circulating NK cells in Behçet's disease, Hasan MS *et al.*[14] reported a significantly reduced number of CD 56+ NK cells ($P<0.05$). Recently, Hus *et al.*[15] investigated mean platelet volume (MPV) levels in 28 progressive diffuse large B-cell lymphoma patients and reported that absolute counts of CD3-/CD16+, CD56+ NK cells were significantly lower than that of controls ($P<0.05$). Consistent with previous studies, Ichikawa *et al.*[16] found that the number of circulating NK cells was fewer in patients with Sjögren's syndrome than in the healthy controls ($P<0.05$). In addition, Zheng *et al.*[17] reported that patients with active Crohn's disease had a higher number of peripheral NK cells than patients with active ulcerative colitis ($P<0.01$). All of the studies mentioned including ours suggest that the number of circulating NK cells may vary according to disease.

Environmental, genetic, and immunological factors are believed to affect the etiopathogenesis of CD.[18] As in other autoimmune diseases, the role of NK cells in CD has been investigated, but most of these studies were performed in adults.[19,20] A study reported that in adult patients with CD, the mean absolute number of NK CD3-/CD56+ cells in peripheral blood [340 (SD 50) cells/mL] was significantly lower than in the controls [360 (SD 80)

cells/mL].[4] In addition, another study found that a gluten-free diet in celiac patients led to an increase in NK cells.[21] Consistent with those results, a previous study claimed that deficiency of NK cells may cause the loss of immunological tolerance in patients with CD.[22] On the other hand, Dunne *et al.*[23] reported that circulating innate lymphocyte populations, including NK CD56+ cells and invariant NK T cells, were significantly decreased in patients with CD, but not in celiac children. Our study results were inconsistent with those of a previous study, with a possible reason may have been their small number of subjects (n=22).[23]

The reduction of absolute number of NK cells in circulation is generally parallel to a reduction in NK cell cytotoxicity. Low NK cells and cytotoxicity could lead to defective control of ongoing autoimmune responses.[24,25] Few studies have investigated the relationship between NK cells and cancer in celiac disease. A previous study reported that the decrease of NK cells may increase the prevalence of malignancy in untreated celiac adult patients.[26] Although celiac children in our study did not comply with the gluten free diet (GFD), no malignant disease was encountered. However, it is necessary to keep this risk in mind in untreated celiac patients.

For evaluating compliance to GFD, tTG-IgA and EMA- IgA levels have been widely used in the celiac patients.[2] We found no correlations between NK cell counts and tTG-IgA, tTG-IgG, or EMA-IgA levels. Hence, these markers may be inappropriate for monitoring compliance to the GFD, especially in children. We could not find a correlation study between these parameters in the pediatric medical literature.

There were some limitations in this study. As there have been relatively few studies evaluating the number of peripheral blood NK cells in children with CD, we compared our findings to only a small number of studies. A second limitation was that we did not evaluate NK cell activity in the intestinal tissue.

In conclusion, to date, few researchers have focused on absolute numbers of circulating NK cells in children with celiac disease. In our study, celiac patients have significantly lower absolute numbers of circulating NK cells than controls. More studies will be required on the role of NK cells in order to elucidate the pathogenesis of celiac disease.

Conflict of Interest

None declared.

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Original Article

Ouvrier's Modified Mini Mental State Examination as a screening test for cognitive impairment in pediatric epilepsy

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Abstract

Background Epilepsy may affect children's development, including their cognitive function. The prevalence of cognitive impairment in epilepsy patients is quite high. *Weschler Intelligence Scale for Children* (WISC) takes a long time to administer and is expensive, so a simpler screening tool for cognitive evaluation is needed in pediatric epilepsy patients.

Objective To assess the diagnostic value of *Ouvrier's Modified Mini Mental State Examination* (MMSE) for detecting cognitive impairment in children aged 8-11 years with epilepsy.

Methods This diagnostic study was conducted in December 2018 to February 2019 at Cipto Mangunkusumo and Fatmawati Hospitals in Jakarta. Data were collected with purposive sampling of children with epilepsy aged 8 to 11 years. Cognitive function was assessed by *Ouvrier's Modified* MMSE and WISC. *Ouvrier's Modified* MMSE was compared to WISC as the gold standard. Results were analyzed using a 2x2 table.

Results The prevalence of cognitive impairment in 8 to 11-year-old epilepsy patients was 72.9%. *Ouvrier's Modified* MMSE had 83% sensitivity, 85% specificity, 94% positive predictive value, 65% negative predictive value, and 83% accuracy.

Conclusions *Ouvrier's Modified* MMSE has good diagnostic value, thus it may be useful for early detection of cognitive impairment in pediatric epilepsy. **[Paediatr Indones. 2020;60:137-41; doi: http://dx.doi.org/10.14238/pi60.3.2020.137-41]**.

Keywords: *pediatric epilepsy; MMSE modified Ouvrier; cognitive function*

Childhood epilepsy is known to impact cognitive development.[1,2] There is a high prevalence of cognitive impairment in children with epilepsy, even in patients without brain lesions.[3] The presence of cognitive impairment has a significant effect on the quality of life for children with epilepsy through its impact on learning and social skills.[4]

Cognitive function is assessed by measuring thinking ability, according to age and stage of development. The most widely accepted gold standard measure of cognitive function is the *Wechsler Intelligence Scale for Children* (WISC), from which the intelligence quotient (IQ) can be computed. However, the WISC requires administration by a trained professional, most often a psychologist, is time-consuming and costly.

Ouvrier's Modified Mini Mental State Examination (MMSE) is a reliable alternative to screen for

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cognitive impairment in children.[5-7] In Indonesia, it has been validated in children aged 8 to 11 years in three elementary schools in Bandung, West Java.[8] At Cipto Mangunkusumo Hospital in Jakarta, *Ouvrier's Modified* MMSE revealed a 74.2% prevalence of cognitive impairment in epilepsy patients aged 8 to 11 years.[9] However, the sensitivity and specificity of the Indonesian version have not been validated against a gold standard test in children with epilepsy. This study was conducted to determine the diagnostic value of *Ouvrier's Modified* MMSE in children aged 8 to 11 years with epilepsy.

Methods

We conducted a cross-sectional study from December 2018 to February 2019 in the Pediatric Neurology Outpatient Clinic of Cipto Mangunkusumo Hospital and Fatmawati Hospital, Jakarta. We included children aged 8 to 11 years with epilepsy identified from medical records. Children with visual impairment and/or severe hearing loss, new patients who had seizures within one day of the examination, those who did not complete the MMSE questionnaire, and patients with comorbidities affecting cognitive function, such as cerebral palsy and Lennox Gastaut syndrome, were excluded.

All subjects were tested using the *Ouvrier's Modified* MMSE. The MMSE consists of seven cognitive domains: orientation, registration, attention and calculation, recall, language, and visuospatial function. In children aged 8-11 years, *Ouvrier's Modified* MMSE had a total score of 35 and it had been translated to bahasa. Children with scores below the cut-off value of 26 were considered to have cognitive impairment. All subjects who had been tested using *Ouvrier's Modified* MMSE were then subjected to a gold-standard cognitive examination using the *Wechsler Intelligence Scale for Children* (WISC) administered by a psychologist. The study protocol was approved by the Medical Research Ethics Committee, Universitas Indonesia Medical School.

Data analysis was done using *SPSS version 20.0*. We constructed a 2x2 table of the results of both tests and calculated the sensitivity, specificity, and predictive values of *Ouvrier's Modified* MMSE.

Results

From the medical record search, we identified 155 epilepsy patients aged 8 to 11 years in Cipto Mangunkusumo Hospital (130 patients) and Fatmawati Hospital (25 patients). Of these, 42 subjects in Cipto Mangunkusumo Hospital and 6 subjects in Fatmawati Hospital completed the study (**Figure 1**). Subjects' characteristics are shown in **Table 1**.

Mean MMSE sub-test scores are presented in **Table 2**. Mean scores were highest in the language and orientation sub-tests and lowest in the attention and calculation sub-test. On WISC testing, mean full IQ was 76.08 (SD 19.99), mean verbal IQ was 79.19 (19.48), and mean processing IQ was 75.85 (SD 20.10). According to the gold standard WISC test, 35 subjects had cognitive impairment. Of these, 29 had MMSE scores below the cut-off of 26. Out

Table 1. Subjects' characteristics

Characteristics	(N=48)
Gender, n (%)	
Male	27 (56.2)
Female	21 (43.8)
Age, n (%)	
8-9 years	14 (29.2)
9-10 years	7 (14.6)
10-11 years	27 (56.2)
Age at onset of epilepsy, n (%)	
≤ 5 years	24 (50)
> 5 years	24 (50)
Types of onset, n (%)	
Focal onset	19 (39.5)
General onset	29 (60.5)
Types of epilepsy, n (%)	
Intractable	18 (37.5)
Not intractable	30 (62.5)
Type of therapy, n (%)	
Monotherapy	28 (58.4)
Polytherapy	20 (41.6)

Table 2. Mean and median MMSE sub-test results

MMSE	Mean (SD)	Median (range)
Orientation	6.23 (2.95)	7 (1-10)
Registration	2.88 (0.49)	3 (0-3)
Attention & calculation	3.44 (3.13)	3 (0-10)
Recall	2.73 (0.74)	3 (0-3)
Language	8.02 (1.66)	9 (3-9)

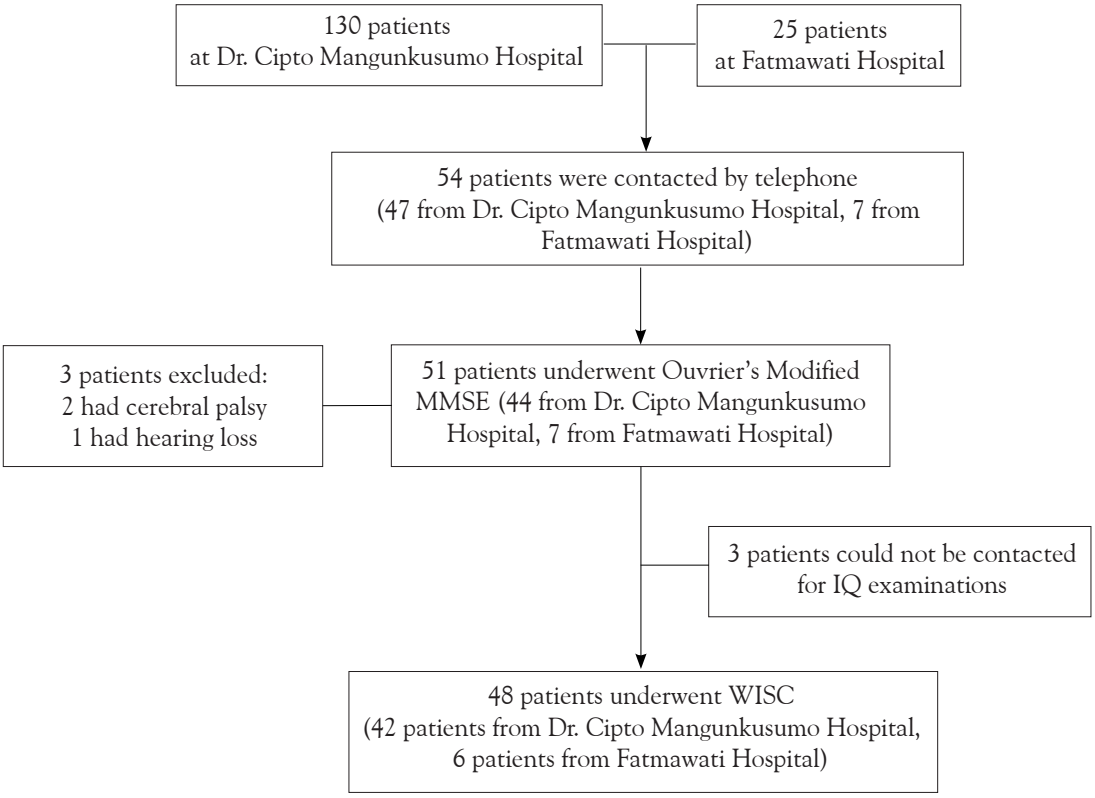

Figure 1. Subject recruitment flow chart

Table 3. Determination of cognitive impairment using *Ouvrier's Modified* MMSE *vs.* WISC

		Gold standard (WISC)		
	Cognitive impairment	Yes	No	Total
Ouvrier's Modified MMSE	Yes	29	2	31
	No	6	11	17

of 13 subjects without cognitive impairment, 11 had normal MMSE scores (**Table 3**). Ouvrier's Modified MMSE had a sensitivity of 83%, specificity of 85%, positive predictive value of 94%, negative predictive value of 65%, and an accuracy of 83%.

Discussion

Ouvrier's original study was conducted in 117 children aged 4-15 years, 71% of whom were male.[5] In a Bandung, West Java, Indonesia study involving 100 students aged 8-11 years, males comprised 55%.[8] Another study in Jakarta using *Ouvrier's Modified MMSE* found cognitive impairment in 74.29% of children with epilepsy aged 8-11 years. The study also found a frequency of seizures > 10 times, the statistical risk of 59.5 times greater for increasing cognitive numbers. In addition, patients receiving antiepileptic drug polytherapy had a greater increase in improved cognition than patients receiving monotherapy.[9]

In our study, the proportion of children with cognitive impairment was quite high, 35 subjects (72.9%). Mean WISC Full IQ test score for all subjects was 76.08 (SD 19.99). Treatment plans for cognitively impaired patients include specialized therapy to help children adapt and develop with their conditions. The subjects were advised to attend specialized schools. Cognitively impaired children also need counseling, as well as evaluations of the family system and the

influence of this cognitive disorder on the family.

The MMSE sub-tests with the highest mean scores were language [mean 8.02 (SD 1.66)] and orientation [mean 6.23 (SD 2.95)]. This result is consistent with the existing view that brain development during middle childhood is characterized by growth of the frontal lobe and maturation of the temporal lobe, two structures that play an important role in the orientation and language processes.

The dose and timing of stimulation given to a child determine whether the stimulus will be maintained as an experience. Such experiences play an important role in synaptogenesis. Adequate, repetitive, and consistent stimulation increases the branching of dendrites and proliferation and stabilization of synapses.[10-13]

In our study, the attention and calculation sub-test had the lowest mean MMSE score [mean 3.44 (SD 3.13)]. Calculation and backward spelling require more complex work and involve both cerebral hemispheres, especially in the counting process. Counting skills require a more complicated interaction between the language, visuospatial, and executive centers to maintain attention and working memory. These functions require communication between several brain areas, such as the dorsolateral prefrontal portion, the frontal lobe, the inferior parietal lobe, and the angular gyrus of the corpus callosum. Logical reasoning ability begins to develop at the age of 8-12 years. The development of working memory in the frontoinsular-temporal networks continues into adolescence, and the capacity of this system is associated with prefrontal-parietal connectivity. The dorsolateral prefrontal area of the frontal lobe is closely associated with the ability to analyze and solve problems. Immature development of these areas is also affected by a lack of experience and improper learning processes, which will manifest as difficulties in counting and backward spelling.[10-14] Since children who have these difficulties tend to avoid mathematical learning, they tend to be understimulated in this area.[15-17] These reasons may explain the low sub-test score in counting and backward spelling in our subjects.

The sensitivity (83%) and specificity (85%) of *Ouvrier's Modified MMS*E in our study suggests that this tool is potentially useful as a screening test for cognitive function abnormalities in children of this age group. A meta-analysis of the diagnostic performance of MMSE in detecting dementia and mild cognitive impairment in primary care reported a sensitivity of 78.4% and specificity of 87.8%.[18] Another study which used MMSE to assess cognitive function in children aged 3-14 years with encephalopathy reported a sensitivity of 35% and specificity of 100%. Re-testing four days after the first administration showed a sensitivity of 68% and specificity of 100%.[6] Despite slight differences between studies, all studies to evaluate the performance of MMSE have reported that this test can be used as a screening tool to assess cognitive function in normal children, as well as in children with epilepsy. The Indonesian version of *Ouvrier's Modified MMSE* requires only 5-10 minutes to administer and can be done on a regular outpatient follow-up visit at no additional cost, while the WISC may take up to 60 minutes and incurs a significant cost.

In conclusion, the Indonesian version of *Ouvrier's Modified MMSE* has a reasonably high sensitivity and specificity to predict cognitive impairment in 8 to 11-year-old children with epilepsy, therefore, it may serve as a useful screening tool.

Conflict of Interest

None declared.

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Original Article

Association of BMI measurements to waist circumference and waist-to-height ratio in overweight and obese children

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Abstract

Background Early monitoring of visceral fat is important to prevent the worsening of obesity in children. In recent years, waist circumference (WC) and waist-to-height ratio (WHtR) measurements have gained attention as an anthropometric indexes for obesity in children. They are an easy-to-use, inexpensive, specific to visceral fat and safe monitoring methods for children. International reference values, however, do not exist for any of the two measures to determine obesity in children.

Objective To compare WC and WHtR to body mass index (BMI) status in overweight and obese children.

Methods This cross-sectional study included overweight and obese children aged 10-12 years from four primary schools in Semarang, Central Java. Subjects underwent anthropometric measurements including weight, height, and waist circumference. Subjects were classified as obese (≥P95) or overweight (P85≤P<P95) using BMI percentiles according to age and sex. Chi-square test was used to assess for associations between categorical variables and multivariate logistic regression analysis was used to identify a dominant variable.

Results Forty-two obese and 23 overweight children were studied. Children with higher values of WC (PR=1.879) and WHtR (PR=8.352) had a higher prevalence of having higher BMI status (obese). Using multivariate analysis, WHtR was the more dominant variable associated with BMI status, compared to WC.

Conclusion Higher WC (cut off P90) and WHtR (cut off 0.5) have a significant associations with greater obesity children aged 10-12 years. Compared to WC, WHtR is a stronger predictive factor for obesity. **[Paediatr Indones. 2020;60:131-6; doi: http://dx.doi.org/10.14238/pi60.3.2020.131-6]**.

Keywords: *waist circumference; waist-to-height ratio; obesity; children*

Obesity is one of the leading global mortality risks.[1] Worldwide obesity has nearly tripled since 1975.[2] Childhood obesity is associated with a higher chance of obesity in adulthood. The degree of obesity in adulthood is associated with several metabolic and cardiovascular complications.[3] The prevalence of overweight and obesity in 5 to 19-year-old children and adolescents increased from 4% in 1975 to 18% in 2016.[4] Indonesia itself in 2013, had the highest prevalence of overnourishment in aged 5-12 years group which is 18.8%, with overweight 10.8% and obese 8%.[5]

Obesity is defined as abnormal or execessive fat accumulation that may impair health.[4] The WHO noted that the site of fat accumulation makes a difference in terms of increasing health risk. People with visceral fat are more likely to have chronic inflammation and associated with a higher chance of serious illness than those with subcutaneous fat.[6,7] Early monitoring of visceral fat is important to prevent

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the worsening of obesity in children. Although BMI has been widely used to measure obesity, it has limitations, in that BMI does not indicate body composition or the pattern of fat distribution. Magnetic resonance Imaging (MRI) is considered to be the gold standard to assess visceral fat, but this technique is expensixe and is impractical for routine clinical settings or large-scale studies.[8,9] In recent years, waist circumference (WC) and waist-to-height Ratio (WHtR) measurements have gained attention as an anthropometric indexes for obesity in children.[9,10] They are an easy-to-use, inexpensive, accurate and safe monitoring methods for children. International reference values, however, do not exist for any of the two measures to determine obesity in children. The objective of this study was to compare WC and WHtR to BMI status in overweight and obese children aged 10-12 years.

Method

This cross-sectional study was done to evaluate the usefulness of WC and WHtR to assess for obesity in children. This study was conducted from April to August 2019 at four primary schools in Semarang, Central Java, and employed a consecutive sampling method. The inclusion criteria were overweight and obese children by BMI aged 10 to 12 years whose parents or guardians allowed them to undergo anthropometric measurements such as weight, height and waist circumference. The exclusion criteria were students who were taking long-term medication (ec. corticosteroid), or were normal or underweight by BMI.

This study was approved by the Research Ethics Committee of Dr. Kariadi General Hospital Semarang. Subjects' parents or guardians provided written informed consent. After informed consent was obtained, students underwent history taking for collection of basic demographic data, as well as their history of drug use. Students who met the inclusion criteria underwent anthropometric measurements of weight, height and waist circumference.

Body weight was measured in kilograms using Body Impedance analysis *Tanita® BC 601*, with a measurement scale up to 270 kg and precision of 0.1 kg. Height was measured in centimeters with to the nearest 0.1 cm. Waist circumference was measured with a non-elastic measuring tape at the midpoint between adjacent last ribs and the peak of the iliac crest, at the end of normal expiration, with the subject standing upright, feet together, and with no clothes covering, to the nearest 0.5 cm. To minimize measurement bias, measurements were done twice then averaged, if the difference was less than 1 cm. If the difference exceeded 1 cm, the measurement was repeated.

Subjects' BMI were calculated using the weight and height measurements and classified according to age and sex as obese for $\geq P_{95}$ and overweight for $P_{85}<P<P_{95}$.[11] For WC, subjects were classified as WC $\geq P_{90}$ or WC$<P_{90}$.[12-16] Waist circumference percentiles for children were based on data from CDC.[17] WHtR was calculated using WC and height measurements, and classified as WHtR ≥ 0.5 and WHtR <0.5.[9,10,18-21] For height, subjects were classified as tall ($\geq P_{95}$), normal ($P_5 \leq P < P_{95}$) or short ($<P_5$), using criteria and percentiles from CDC.[17]

Data were analyzed using *SPSS statistical software version 23.0* with a significance level of $P<0.05$ and 95% confidence interval (CI). Descriptive data were shown in percentage, mean and standard deviation. Fisher's exact and Chi-square tests were used to analyze for possible associations between WC and WHtR with BMI status. Multivariate logistic regression was used to further analyze for a dominant variable.

Results

Of 88 students at four primary schools who met the inclusion criteria, 20 students refused to join the study. Due to parent's objections, the Tanner staging examination could not be performed. Of the 68 students who were willing to join, 3 students had attained menarche. Hence, the final sample comprised 65 students. Subjects' mean age was 10.75 years. The demographic characteristics of subjects are shown in **Table 1**.

Twenty-four subjects (36.9%) had WC $\geq P_{90}$. Chi-square test revealed a significant correlation between WC and BMI status ($P<0.001$), as shown in **Table 2**. The prevalence ratio (PR) value was 1.879 (95%CI 1.343 to 2.630), indicating that children with WC $\geq P_{90}$ had a 1.879 times increased risk of being

obese (by BMI) compared to those with WC <P_{90}.

Fifty-four subjects (83.1%) had WHtR ≥0.5. Chi-square test revealed a significant correlation between WHtR and BMI status (P<0.001), as shown in **Table 3**. The PR value was 8.352 (95%CI 1.281 to 54.449), indicating that children with WHtR ≥0.5 had a 8.352 times increased risk of being obese by BMI compared to those with WHtR <0.5.

Fifty-six subjects (86.1%) had normal height (P5≤P<P95). Chi-square test revealed that height and BMI status did not have a significant correlation (P>0.05), as shown in **Table 4**.

Multiple logistic regression analysis revealed that WHtR (P=0.011) was the more dominant variable of the two associated with obesity by BMI. The PR value was 17.273 (95%CI 1.942 to 153.664), indicating that the risk of obesity by BMI in children with WHtR ≥0.5 was 17.273 times higher than in children with WHtR <0.5, as shown in **Table 5**.

Table 1. Demographic characteristics of subjects

Characteristics	(N=65)
Gender, n(%)	
Male	42 (64.6)
Female	23 (35.4)
Age, n(%)	
10 years	28 (43.1)
11 years	25 (38.5)
2 years	12 (18.5)
Weight, kg	
Mean (SD)	53.3 (10.9)
Median (range)	53.8 (34.2-91.2)
Height, cm	
Mean (SD)	144.5 (7.8)
Median (range)	144 (126-164)
Height category, n(%)	
Tall	4 (6.1)
Normal	56 (86.2)
Short	5 (7.7)
Body mass index, kg/m2	
Mean (SD)	25.4 (3.8)
Median (range)	24.65 (19.8-34.7)
BMI status, n(%)	
Obese	42 (67.7)
Overweight	23 (32.3)
WC, cm	
Mean (SD)	82.4 (10.6)
Median (range)	83 (60-110.5)
WC category, n(%)	
≥P90	24 (36.9)
<P90	41 (63.1)
WHtR	
Mean (SD)	0.57 (0.07)
Median (range)	0.572 (0.43-0.74)
WHtR category, n(%)	
≥0.5	54 (83.1)
<0.5	11 (16.9)

Discussion

We found a higher prevalence of obesity in boys (64,6%) than girls (35,4%). A previous study reported that the higher prevalence of obesity in boys was due to their higher average energy and carbohydrate intake compared to that of girls.[22] In addition, children in this age group become more aware of their bodies.[22,23] Thus, girls in this age group may start to restrict their diet.[22]

Our study showed a significant association between WC ≥P90 and obesity in children (95%CI 1.343 to 2.630; P<0.001). Children with WC ≥P_{90} had a 1.879 times increased risk of being obese (by BMI) compared to those with WC <P_{90}. A previous study in Norway also found a significant association between WC and BMI.[24] A person with obesity had excess fat accumulation on their body that will continue to fill possible space in the body such as under the skin or between visceral organs.[25] Based on location, fat can be classified into visceral/central and subcutaneous/peripheral.[26] Subcutaneous fat accumulation occurs during excess energy intake (high-caloric diet) with limited energy expenditure (physical inactivity). When the subcutaneous storage

Table 2. Association between WC and BMI status

Waist circumference	Obese n (%)	Overweight n (%)	PR	95%Ci	P value
≥P_{90}	22 (52.4)	2 (8.7)	1.879	1.343 to 2.630	<0.001
<P_{90}	20 (47.6)	21 (91.3)			
Total	42 (64.6)	23 *35.4)			

Table 3. Association between WHtR and BMI status

WHtR	Obese n (%)	Overweight n (%)	PR	95%Ci	P value
≥0.5	41 (97.6)	13 (56.5)	8.352	1.281 tp 54/449	<0.001
<0.5	1 (2.4)	10 (43.5)			
Total	42 (64.6)	23 (35.4)			

Table 4. Association between height and BMI status

WHtR	Obese n (%)	Overweight n (%)	PR	95%Ci	P value
Tall	4 (9.5)	3 (13)	0.372	0.68 to 2.049	0.123
Normal	37 (88)	19 (82.6)			
Short	1 (2.5)	1 (43.4)			
Total	42 (64.6)	23 (35.4)			

Table 5. Multivariate logistic regression analysis of WC and WtHR with BMI status

Variables	PR	95%CI	P value
WC	6.368	1.252 to 32.402	0.026
WHtR	17.273	1.942 to 153.664	0.011

capacity is exceeded or its ability to generate new adipocytes is impaired, fat begins to accumulate in areas outside the subcutaneous tissue, typically in visceral areas.[27] Waist circumference measurement can be used to determine fat mass and predict visceral fat. An Australian study noted that WC was a significant predictor of intraperitoneal abdominal adipose tissue mass (IPATM) and retroperitoneal adipose tissue mass (RPATM) predictor.[15] As such, high visceral fat measured by WC was typically found in subjects with higher level of obesity.

We also found a significant association between WHtR ≥0,5 and obese in children (95%CI 1.281 to 54.449; P<0.001). Children with WHtR ≥0.5 had a 8.352 times increased risk of being obese by BMI compared to those with WHtR <0.5. A Bangladesh study also showed a significant positive association between WHtR and BMI.[28] Height varies among population, as well as in children of varies ages. Height impacts the distribution of fat, where an increase of height could be followed by an increase of WC. This statement is in agreement with the CDC reference for anthropometric measurement that height and WC showed an increasing trend with age.[17] Hence, WHtR gained attention as a measurement of visceral fat. WHtR had a strong correlation with visceral fat in Chinese study (r=0.868). Our subject showed that high fat accumulation are not accompanied by higher height, since obese (88%) and overweight (82.6%) mostly had a normal height. When a child's calorie intake is more than sufficient to achieve linear growth, the excess would then be stored as subcutaneous and visceral fat.[29] Similarly, a previous Indonesian study reported that an increase of BMI was not followed by an equal of height increase. The average height in girls was not significantly different between those who were obese and normal weight.[30] Therefore, high visceral fat measured by WHtR could be found in subjects with higher level of obesity. This measurement may allow the same boundary values for children and adults, and is not affected by age and ethnicity.[10] Both measurements had significant associations with BMI status, therefore we performed a multivariate analysis. Compared to WC, WHtR was the more dominant variable associated with BMI status.

Our study provides evidence that WC (cut off P_{90}) and WHtR (cut off 0.5) have a significant association with BMI status in children aged 10-12 years. Compared to WC, WHtR is a stronger predictive factor for obesity. The limitations of our study were using consecutive sampling that could result a selection bias, not having normoweight subject as an inclusion could result information bias and using a questionnaire as a subjective measurement to exclude subjects who has reached puberty could result a measurement bias. Further studies are needed using random sampling, and an objective measurement such

as Tanner staging to accurately assess puberty, also adding normoweight as a sample.

Conflict of Interest

None declared.

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Original Article

Parental knowledge, attitude, and behavioral factors in immunization response following a diphtheria outbreak in children in 2018-2019

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Abstract

Background A diphtheria outbreak was declared at the end of 2017. The outbreak response immunization (ORI) was a key Indonesian government strategy to control diphtheria in three outbreak areas. This strategy was implemented starting December 11, 2018. Parents' positive knowledge, attitude, and behavior regarding diphtheria and the diphtheria ORI may influence the coverage of diphtheria ORI in Indonesia.

Objective To assess for relationships between parental knowledge, attitude, and behavior to coverage of diphtheria ORI in children.

Methods This cross-sectional study was conducted at Kapuk Muara Public Elementary School 03, North Jakarta from November 2018 to August 2019. The respondents were parents of the schoolchildren. Parents filled questionnaires about their knowledge, attitude, and behavior with regards to diphtheria ORI coverage in children.

Results The coverage of diphtheria ORI in children was 61.8%. From 110 respondents, 40.9% of parents had at least sufficient knowledge, 73.8% had good attitude, and 55.5% had good behavior regarding diphtheria and diphtheria ORI. Parental knowledge had no significant association to coverage of diphtheria ORI in their children. However, there were significant relationships between parental attitude and behavior to coverage of diphtheria ORI in their children.

Conclusion The coverage of diphtheria ORI needs improvement. Most parents have sufficient level of knowledge, good attitude, and good behavior towards diphtheria and diphtheria ORI. There is no association between parental knowledge about diphtheria and diphtheria ORI to coverage of diphtheria ORI, but there are significant association of parental attitude and behavior toward diphtheria ORI coverage. **[Paediatr Indones. 2020;60:142-8; doi: http://dx.doi.org/10.14238/pi60.3.2020.142-8]**.

Keywords: *ORI diphtheria; parent's knowledge; attitude, behavior*

Diphtheria is caused by Corynebacterium diphtheriae.[1] The bacteria produce endotoxins that cause tissue necrosis, airway obstruction, and myocarditis.[2-5] The Indonesian Ministry of Health recorded 591 cases of diphtheria in 20 provinces in Indonesia by the 48th week of 2017 and declared diphtheria to be an outbreak.[6] *The Diphtheria Outbreak Response Immunization* (ORI) was one government strategy to control the outbreak through additional immunizations in affected regions.[2,7]

The World Health Organization (WHO) stated that lack of complete immunization coverage over a long period of time is one of the causes of diphtheria outbreaks.[7] The government has promulgated a mandatory complete basic immunization program since 1974 [*Expanding Program on Immunization*/EPI]. Immunization coverage reached 80% up until 1990.[8] Diphtheria immunization is one of the mandatory vaccinations that every baby or child in Indonesia should receive.[9]

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The knowledge and behavior of parents about immunization are major factors in children's completion of immunizations. A study in Iraq reported a positive correlation between immunization coverage and parental knowledge and behavior of immunizations.[10] In addition, a study in Malaysia showed that interventions to provide information about immunizations increased parental knowledge on the topic.[11]

The Indonesian Ministry of Health implemented the diphtheria ORI since December 11, 2018, in three outbreak areas (Jakarta, Banten, and West Java provinces). Knowledge, attitude, and behavior of parents may have an affect on the coverage of diphtheria ORI in Indonesia. Until January 11, 2018, the average coverage of diphtheria ORI was 68.36% of the total target of 7.9 million persons. In early January 2018, no additional areas reported diphtheria outbreaks.[6,7,12-14] As such, we aimed to assess the coverage of the diphtheria ORI in one primary school, and analyze for associations to parental knowledge, attitude, and behavior on immunizations.

Methods

Subjects' characteristic in this study were divided into 3 categories based on their socioeconomic level, categorized as middle-lower if subjects' average monthly income was below 2,600,000 IDR, intermediate 2,600,000-6,000,000 IDR, and middle-upper if above 6,000,000 IDR.[15-16] Subjects' knowledge was classified into 3 categories; good, sufficient, poor. Subjects' knowledge was categorized as good if the final score in the questionnaire was above 75%, sufficient 56-75%, and poor if below 56%.[17] Subjects' attitude was classified into 2 categories (good and poor) based on the median value of the total points in the questionnaire. It was categorized as good if the final score was more than 9 points and categorized as poor if less than 9 points. Meanwhile, the subjects' behavior was classified into 2 categories (good and poor) based on the median value of the total points. Categorized as good if the final score was more than 7 points and poor if less than 7.[18] Diphtheria ORI coverage was categorized as complete if children received additional diphtheria immunization. Additional diphtheria immunization in this study meant outbreak-related additional doses in children who had complete childhood basic immunization; were classified as complete if they received 3 additional doses in accordance with the diphtheria ORI provision and "incomplete" if they only received 1 or 2 additional doses.[19]

This cross-sectional study was conducted from November 2018 to August 2019 at Public Elementary School 03, Kapuk Muara, North Jakarta. Subjects were parents of the students. Subjects were chosen by simple random sampling. Inclusion criteria were parents (aged 17-55 years) who had children aged 1-19 years and knew about diphtheria immunization as a response to the outbreak in Indonesia. Exclusion criteria were parents who refused to participate, could not communicate properly (had mental disability, or hearing and speech impairment), or did not know about diphtheria immunization or the diphtheria ORI. Parents who removed themselves when the study was in progress were considered to have dropped out.

The minimum required sample size was estimated to be 96, with an additional 15% added to anticipate dropping out. The sample size was, therefore, 110 subjects. This study was approved by the Research Ethics Committee of Universitas Katolik Indonesia Atma Jaya, School of Medicine and Health Sciences. Data were collected from questionnaires filled directly by the subjects with supervision from the researcher. The questionnaires used in this study consisted of questions about parental knowledge (12 questions), attitude (7 questions) towards diphtheria and diphtheria ORI, and behavior (4 questions) towards diphtheria ORI. In questions about knowledge, if the subjects answered correctly then a score of 1 was given and 0 if the answer was wrong. In questions about attitude, if the subjects answered accordingly then a score of 2 was given, 1 if the answer was wrong, and 0 if the subjects answered "do not know". In questions about behavior, if the subjects answered yes or correct then a score of 2 was given, 1 for a no or incorrect answer, and 0 for a "do not know" answer (**Appendix**). The questionnaire was created by the researchers and has been validated to a population that was similar to the target population. Data were analyzed with *SPSS version 23* software, using a non-parametric comparative test with a 95% confidence level. Bivariate data were analyzed using Chi-square test for results with P values < 0.05. A P value of < 0.05 was considered to be statistically significant.

Results

Characteristics of study subjects are shown in **Table 1**. Most study subjects were female (98.2%). Subjects were mostly in late adulthood or 36-45 years old (46.4%) and mostly senior high school graduates (37.3%). The most common socioeconomic level was middle-lower level (66.4%), with only a small proportion in the middle-upper level (4.5%).

Table 1. Study subjects' characteristics

Demographic characteristics	(N=110)
Gender, n (%)	
Male	2 (1.8)
Female	108 (98.2)
Age by group, n (%)	
Late teens (17-25 years)	0 (0)
Early adulthood (26-35 years)	47 (42.7)
Late adulthood (36-45 years)	51 (46.4)
Early elderly (46-55 years)	12 (10.9)
Highest education level attained	
Elementary	38 (34.5)
Junior high	30 (27.3)
Senior high	41 (37.3)
College	1 (0.9)
Socioeconomic Level	
Middle-lower	73 (66.4)
Intermediate	32 (29.1)
Middle-upper	5 (4.5)

Fifty-five (40.9%) subjects had sufficient knowledge and 41 (37.3) had poor knowledge. There were only 24 (21.8%) subjects with good knowledge about diphtheria and diphtheria ORI. Sixty-one (80.9%) subjects had good attitude and 49 (44.5%) had poor attitude. Most study subjects had good behaviour (80.9%), and only 21 (19.1%) had poor behaviour (**Table 2**).

Table 2. Parental knowledge, attitude, and behavior about diphtheria and diphtheria ORI

Variables	(N=110)
Parental knowledge, n (%)	
Good	24 (21.8)
Sufficient	45 (40.9)
Poor	41 (37.3)
Parental attitude, n (%)	
Good	61 (80.9)
Poor	49 (44.5)
Parental behaviour, n (%)	
Good	89 (80.9)
Poor	21 (19.1)

Table 3. Diphtheria ORI coverage

Variables	(N=110)
Diphtheria ORI coverage, n (%)	
Complete (3 doses)	68 (61.8)
Incomplete	
1 dose	26 (23.6)
2 doses	16 (14.5)

Sixty-eight (61.8%) subjects allowed for the administration of the complete (3 doses) additional diphtheria immunizations to their children. Twenty-six (23.6%) subjects allowed for only one dose to their children, and 16 (14.5%) allowed for two doses of additional diphtheria immunizations (**Table 3**).

Bivariate analysis of relationships between parental knowledge, attitude, and behavior about diphtheria and ORI diphtheria to coverage of diphtheria ORI are shown in **Table 4**. Chi-square test

Table 4. Bivariate analysis of parental knowledge, attitude, and behavior about diphtheria and diphtheria ORI to diphtheria ORI coverage

Variables	Diphtheria ORI coverage			P value
	Complete (3 doses) n=68	Incomplete n=42	Total N=110	
Knowledge, n(%)				0.075
Good	18 (75)	6 (25)	24	
Sufficient	30 (66.7)	15 (33.3)	45	
Poor	20 (48.8)	21 (51.2)	41	
Attitude				0.004
Good	45 (73.8)	16 (26.2)	61	
Poor	23 (47)	26 (53)	49	
Behavior				0.047
Good	59 (66.3)	30 (33.7)	89	
Poor	9 (42.9)	12 (57.1)	21	

revealed no significant association between parental knowledge about diphtheria and diphtheria ORI to diphtheria ORI coverage (P=0.075).

Of the subjects with good attitude (61/110; 55.5%), the majority (45/61; 73.8%) gave all 3 doses. Of those with poor attitude (49/110; 44.5%), the majority (26/49; 53%) gave fewer than 3 doses. Chi-square test revealed that good parental attitude about diphtheria and diphtheria ORI had a significant association with complete diphtheria ORI coverage (P=0.004).

More than half the number of subjects who had good behavior gave their children the complete 3 doses of additional diphtheria immunization (59/89; 66.3%). However, quite a few subjects with good behavior did not complete the additional diphtheria immunization (30/89; 33.7%). More subjects with poor behavior did not complete the immunizations (12/21; 57.1%) than those who did complete the 3 doses (9/21; 42.9%). Chi-square test revealed a significant association between good parental behavior about diphtheria ORI and complete ORI diphtheria coverage.

Discussion

Diphtheria ORI coverage in Public Elementary School 03, North Jakarta was 61.8%. This result was far below the Ministry of Health target of 95%. Another study conducted at Mijen Health Center, Semarang, Central Java, also reported low diphtheria ORI coverage, although even low national coverage appeared to reduce transmission and death due to diphtheria.[20] Diphtheria ORI were given in 3 rounds; the number of subjects whose children participated in each round consistently declined. Consistent participation may be influenced by community psychosocial concerns with regards to obedience.[21] The quick and sudden implementation of diphtheria ORI also contributed to the decrease in the number of subjects per round.

The third exclusion criteria was parents that did not know about diphtheria immunization or the diphtheria ORI. We did not find it bias because the inclusion criteria was parents who knew about diphtheria and diphtheria ORI but had poor knowledge about it. We found no association between parental knowledge about diphtheria and diphtheria ORI to coverage of diphtheria ORI. In contrast, a previous study reported a significant strong positive correlation between the two.[22] Another study also had similar results, but the correlation between the two variables was low.[23] Several predisposing factors may have influenced the results, including subjects' formal education level, family socio-economic status, sources of information, and others.[24] A factor that also affected the results is subjects' non-compliance to additional immunization, which was a mandatory for students of 03 Public Elementary School. At the beginning of the study 110 students received the first dose of additional ORI immunization, but only 68 students completed the 3 additional doses as some of the parents complaint with the side effect of the additional immunization.

Parental attitude about diphtheria and diphtheria ORI had a significant association with the coverage of diphtheria ORI in children, similar to a study conducted by Dini *et al*.[20] In addition, parental behavior towards diphtheria ORI had a significant association with the coverage of diphtheria ORI, similar to results by Halimantus *et al*. who found a significant but weak correlation.[25]

In conclusion, the coverage of diphtheria ORI at Public Elementary School 03 needs improvement because the coverage was only 68%, meaning that it has not reached the total target of 7.9 million persons. There is no association between parental knowledge about diphtheria and diphtheria ORI to coverage of diphtheria ORI. Meanwhile, parental attitude about diphtheria and diphtheria ORI have a significant association with the coverage of diphtheria ORI in children and parental behavior towards diphtheria ORI also have a significant association with the coverage of diphtheria ORI.

The strength of this study was that it showed the shortcomings of the diphtheria ORI program, which was still far below the target even though this program had been required by the government and given free of charge. Moreover, there were only few studies conducted on this topic. The lack of this study was that it did not learn more about the causes of the reduction in number of respondents on the second and third dose of ORI diphtheria immunization.

Conflict of Interest

None declared.

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Appendix. Questionnaires

I. Characteristics

1. Name and gender	(M/F)	Code
2. Age	years-old (According to the last birthday)	
3. Highest education level attained	Elementary Junior high Senior high College	1 2 3 4
4. Family's average income	Middle-lower (< 2,6 millionIDR) Intermediate (2,6-6 millionIDR) Middle-upper (> 6 millionIDR)	1 2 3

II. Knowledge about diphtheria and diphtheria ORI

No.	Questions	Yes	No
1	Have you ever heard diphtheria?		
2	Is diphtheria a contagious disease?		
3	Is diphtheria transmitted through the respiratory tract?		
4	Do you know the characteristic of diphtheria?		
5	Do you know how to prevent diphtheria in the environment?		
6	Do you know about diphtheria outbreak in Indonesia?		
7	Do you know the cause of diphtheria outbreak in Indonesia?		
8	Do you know about diphtheria Outbreak Response Immunization/ ORI (mandatory diphtheria immunization programme for children aged 1-19) that conducted by the government?		
9	Do you know that the diphtheria ORI conducted by the government is free of charge?		
10	Do you know when your child should be given the required additional diphtheria immunization?		
11	Do you know the side effects of diphtheria immunization?		
12	Do you know about the post-immunization events (adverse events following immunization)?		

III. Attitude about diphtheria and diphtheria ORI

No.	Questions	Answers	Score
1	In your opinion, is diphtheria dangerous?	Agree Not fully agree Disagree Do not know	1 2 3 98
2	What is the reason you consider diphtheria as a dangerous disease?	Causing death Causing other diseases Interfere children's activity Do not know	1 2 3 98
3	In your opinion, is diphtheria ORI programme important and required?	Agree Not fully agree Disagree Do not know	1 2 3 98
4	In your opinion, who is in charge of providing information about diphtheria ORI?	Both parents Husband/ wife Government Health facilities/ services Do not know	1 2 3 4 98
5	What information about diphtheria immunization that you should know as a parent?	The types of The benefits The side effects and how to handle it Time of administration The amount of dose Place to get immunization Do not know	1 2 3 4 5 6 98
6	Do you still bring your child to be given further doses of diphtheria immunization?	Yes No Do not know	1 2 98
7	What is the reason that make you still bring your child to continue to be given further doses of diphtheria immunization after knowing the side effects?	Required by the government Concerned with the children's health The side effects do not cause any worries Do not know	1 2 3 98

IV.Behavior towards diphtheria ORI

No.	Questions	Answers	Score
1	Have you ever participated in counseling about diphtheria ORI?	Yes No	1 2
2	What is the reason you are taking your child for additional diphtheria immunization?	Self awareness Run the government's programme Following the surrounding community Following the family Following the community leaders (tokoh masyarakat) Others _____	1 2 3 4 5 96
3	Have you ever invited people closest to you or the surrounding community to provide additional diphtheria immunization to their child?	Yes No	1 2
4	Does the person closest to you support you to give your child diphtheria immunization?	Yes No Do not know	1 2 98

V. Diphtheria ORI Coverage

No.	Questions	Answers	Score
1	Has your child been given additional diphtheria immunization?	1 dose 2 doses Complete (3 doses)	1 2 3

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Case Report

Pediatric infective endocarditis initially presenting as hemorrhagic stroke

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Infective endocarditis refers to infection of the heart valves. While its incidence is low, it may cause serious complications. Despite advances in its management and diagnosis, this condition still retains high mortality and significant morbidity. Considerable controversy remains regarding antimicrobial prophylaxis to prevent infective endocarditis in patients with congenital heart disease. Neurologic complications are the second most common complication in patients with infective endocarditis, occurring in approximately 33% of cases.[1] These include encephalopathy, meningitis, stroke, brain abscess, cerebral hemorrhage, and seizures. The vegetation formed as a consequence of endocarditis may dislodge and cause embolization. Vegetation size alone is an unreliable marker for embolization risk, however, size, in addition to location, mobility, infecting agent, and presence of antiphospholipid antibodies have the potential to be prognostic markers. The brain is the most frequent site of embolization. Furthermore, advances in medical approaches have resulted in an increase of patients at risk of endocarditis due to the now common and widely available indwelling intravascular approaches in medicine. In this report, we present a case of infective endocarditis in a child first presenting with hemorrhagic stroke.[1,2] **[Paediatr Indones. 2020;60:166-71; doi: http://dx.doi.org/10.14238/pi60.3.2020.166-71**].

Keywords: *endocarditis; aneurysm; stroke*

The Case

A six-year-old Indonesian boy was referred to our facility with a history of persistent fever that had lasted for two weeks. He had recently been diagnosed with ventricular septal defect, as well as tricuspid, mitral, aortic, and pulmonary regurgitation. The child was being followed up while awaiting surgical correction. Two weeks prior to the current admission, he had been admitted to the hospital with a diagnosis of hemorrhagic stroke. He presented with left-sided hemiparesis with motoric strength of 1/5. Multi-slice computed tomography (MSCT) revealed an intracerebral hemorrhage at the right parietal lobe and an adjacent hyperdense lesion. The patient was treated conservatively and subsequently discharged, but he developed a fever at home. His parents denied any history of illnesses in the family. At the time of presentation to our facility, he was afebrile with stable blood pressure. Glasgow coma scale (GCS) at

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presentation was E4V5M6. Physical examination was significant for a heart rate of 150 beats per minute (bpm) and a grade 3/6 pansystolic murmur at the left lower sternal border. The remaining physical examination findings were within normal limits. Neurological examination revealed a left-sided hemiparesis with motor strength of 3/5 (improved compared to 2 weeks prior) and a positive Babinski reflex. It was concluded that this was a residual neurological deficit. To confirm the diagnosis of a mycotic aneurysm, MSCT and computed tomography (CT) angiography were done. The MSCT and CT angiography showed an intracerebral hemorrhage in the right parietal lobe with an adjacent hyperdense lesion, indicative of a mycotic aneurysm. CT angiography also revealed a mycotic aneurysm adjacent to the periphery of the right middle cerebral artery.

Electrocardiography showed the Katz-Wachtel phenomenon (large biphasic QRS complex in leads V2 to V5) suggestive of biventricular hypertrophy. Peripheral blood count revealed anemia (hemoglobin level 8.1 mg/dL and hematocrit 24 vol%) and an elevated erythrocyte sedimentation rate (53 mm/hour). Rheumatoid factor was positive. A chest X-ray showed an upward-pointing apex and a plethora within both lung fields. Echocardiography showed a perimembranous ventricular septal defect (VSD) 4 mm in diameter, with a trans-VSD gradient of 68 mmHg. Mild tricuspid regurgitation, mild mitral regurgitation, moderate aortic regurgitation, and mild pulmonic regurgitation were also present (**Figure 1**). Vegetations were seen on the anterior mitral leaflet with a dimension of 7-9 x 5-6 mm, on the right coronary cusp with a dimension of 6x8 mm, and on the pulmonary valve with a dimension of 6x7 mm (**Figure 2**). Blood culture using a specimen taken prior to the administration of antimicrobials was negative.

We diagnosed the patient with infective endocarditis and administered a treatment regimen of 1.5 g of intravenous (IV) ceftriaxone once daily, 45 mg IV gentamicin once daily, 6.25 mg IV captopril twice daily, 5 mg oral furosemide twice daily, and 6.25 mg oral Aldactone once daily. Repeat echocardiography done 22 days after initial presentation showed reductions in vegetation size to 4x6 mm on the mitral valve, 2.3x3.1 mm on the pulmonary valve, and 3x5 mm on the right coronary cusp. At the time of presentation, no acute management for stroke was undertaken since only residual deficits remained. The patient was scheduled for rehabilitation after the infective endocarditis (IE) was treated. The patient's clinical course is summarized in **Table 1**.

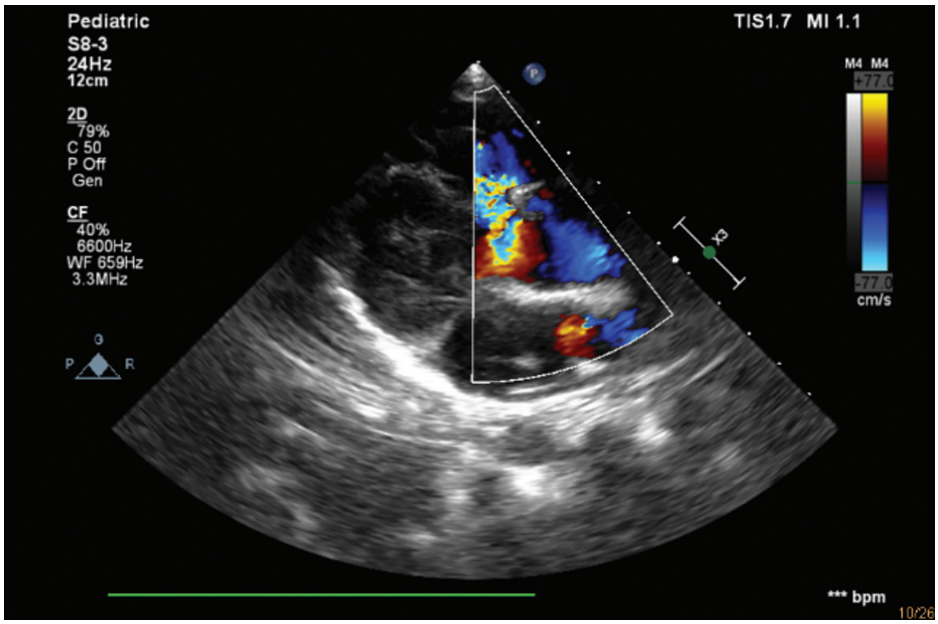

Figure 1. Transthoracic echocardiography with Doppler mode showing tricuspid valve regurgitation

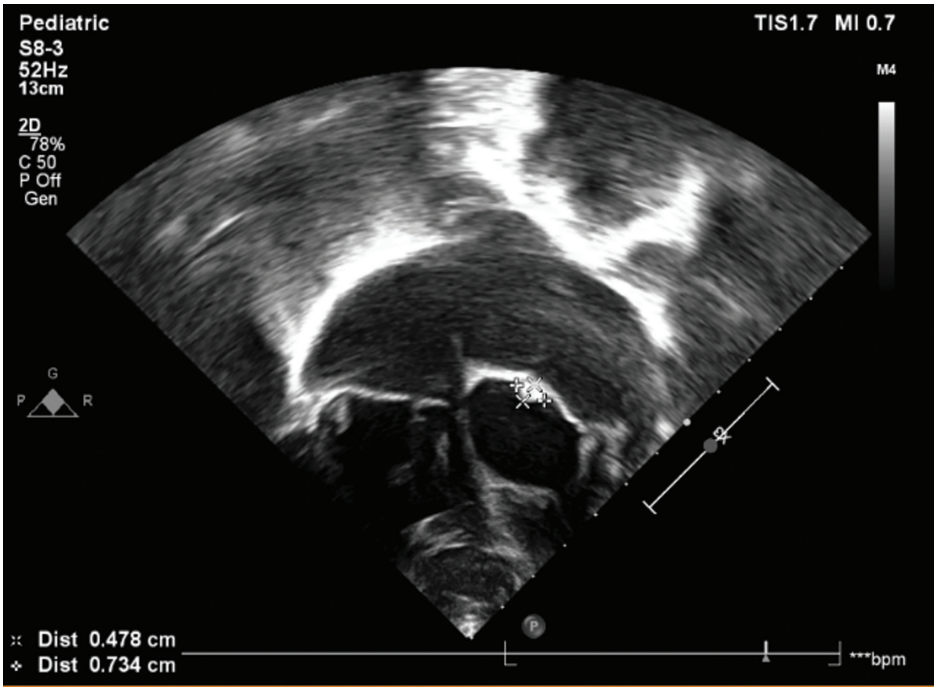

Figure 2. Transthoracic echocardiography showing vegetation on the mitral valve

Table 1. Timeline summary of the patient's progression

Chronology	Progression
One month prior	Diagnosis of VSD, TR, MR, AR, and PR was made
Two weeks prior	Hemorrhagic stroke; experienced fever after discharge
On admission	Infective endocarditis, confirmation of previous CHD diagnosis, residual hemorrhagic stroke, and confirmation of mycotic aneurysm
22 days after admission	Repeat echocardiography showed reductions in vegetation sizes

AR=aortic regurgitation, CHD=congenital heart disease, MR=mitral regurgitation, TR=tricuspid regurgitation, VSD=ventricular septal defect, PR=pulmonary regurgitation

Discussion

The pathogenesis of IE is multifactorial and is the result of complex interactions between bloodstream pathogens, matrix molecules, and platelets at sites of endocardial cell damage.[3] Furthermore, a substantial portion of the disease's clinical manifestations arises from the host's immune response to the infecting microorganisms.[3]

Difference in pressure of chambers of the heart causes turbulence in blood flow, which will inturn predispose to the occurrence in non-bacterial thrombotic endocarditis (NBTE). This turbulence causes trauma in the endothelial and valvular tissues, which will generate platelet and fibrin deposition and finally result in NBTE. This accumulation of platelet and fibrin, in the presence of bacteremia, will be colonized by circulating bacterias and will result in IE.[3]

As mentioned, bacteremia occurs in the pathogenesis of IE. These microbes may originate from sites such as mucosal surfaces, gingival crevices arounnd the teeth, the oropharynx, GI tract, urethra, and vagina.[3] The occurrence of trauma to these sites can release a variety of microbial species transiently into the bloodstream. Transient bacteremia caused by viridans-group streptococci and other oral microflora commonly occur in association with dental extractions

or other dental procedures or with routine daily activities such as toothbrushing.[3] The frequency and intensity of the resulting bacteremia are believed to be related to the nature and magnitude of the tissue trauma. Also, the microbial species entering the circulation depend on the unique endogenous microflora that colonizes the particular traumatized site.[3] Microbiological diagnosis is the most important step in managing IE. In 1 to 2.5% of all IE cases, no microorganisms are isolated in blood cultures, leading to delayed diagnosis and treatment, as well as significant effects on clinical outcomes.[4]

The clinical presentation of this condition has also changed.[5] With the ever-increasing use of antibiotics, the classic IE presentation is generally no longer in the initial findings of patients with the disease. Although far less frequently seen nowadays, classical findings of IE such as Janeway lesions and Roth spots could still be invaluable clues leading to earlier treatment.[5]

Advances in health care delivery have raised the concern of antimicrobial resistance, and the resulting altered epidemiology of infective endocarditis.[6] Methicillin-resistant *Staphylococcus aureus* (MRSA) has been encountered internationally as a relatively common cause of IE, with patients having the distinctive characteristic of persistent bacteremia.6 Despite a higher rate of persistent bacteremia, there is not much difference in mortality between MRSA- and methicillin-sensitive Staphylococcus aureus (MSSA)-infected patients.[6] This finding is believed to be due to the overall high mortality of *S. aureus* IE (regardless of the antimicrobial resistance profile of the infecting pathogen). It is important to note that approximately 20% of patients with MRSA IE developed their infection in the absence of identifiable health care contact.[6]

The differential diagnosis and evaluation of acute stroke in the pediatric population should be based on the understanding that the probability of a non-atherosclerotic etiology is much higher than the adult population.[7] Thus, it is prudent to be aware of the broad and appropriate differential diagnoses for pediatric patients who present with acute ischemic stroke.[7]

Transthoracic echocardiography (TTE) remains an indispensable tool for the diagnosis of IE. Transesophageal echocardiography (TEE), however, remains more sensitive in detecting lesions <1 mm in size.[8] It also poses as an alternative in patients using multiple devices on the chest, such as in intensive care units. However, TTE remains the simplest approach compared to TEE.[9] Anterior mitral leaflet (AML) vegetations remain one of the most common echocardiographic findings in relation to IE, as we noted in our patient.[8,9]

An infectious cerebral aneurysm in IE patients suggests that vascular tissue is friable and vulnerable.[10] Intracranial aneurysm arises from either septic embolism of the vasa vasorum or from subsequent bacterial dissemination spreading from a septic embolism of occluded vessels. Infectious aneurysms can be formed anywhere in the brain arteries, but the distal branches of the middle cerebral artery are the most commonly susceptible, consistent with the findings in our patient.[10] Clinical symptoms are highly variable and include neurological disturbance, headache, confusion, and seizures.[11]

Studies have shown that at least 50% of intracranial aneurysms due to infection decrease in size and are eliminated by the administration of effective antibiotics.[10] Appropriate antibiotic treatment is mandatory for all patients with infectious intracranial aneurysms.[10] Some cases exhibit enlargement of vegetation size despite ongoing antimicrobial therapy of the infection, hence, repeated evaluation using MR and CT angiography is of utmost importance. A Japanese study showed that MRI was superior to CT.[12]

A study of intracranial infected aneurysms complicating endocarditis found that the most significant factor for treatment consideration was whether an aneurysm had ruptured.[13] Treatment-related mortality was higher in patients with ruptured aneurysms than in patients with unruptured aneurysms (24 *vs.* 9%)[13] Among patients with ruptured aneurysms, mortality was higher in those treated with antibiotics alone compared with those treated with both antibiotics and surgery (49 *vs.* 12%).[13] Unruptured mycotic aneurysms are generally treated with antibiotics alone, however, whenever possible, ruptured aneurysms should be managed with a combination of antibiotics and surgery.[14] Endovascular approaches are increasingly being used in such cases.[14]

Our suspicion of infective endocarditis was based on our patient's prolonged fever in the face of

congenital heart disease. He had also been diagnosed with hemorrhagic stroke 2 weeks prior, and at his presentation, the neurological deficits had improved, so he required no immediate post-stroke management. We decided to further explore the cause of the fever by performing CT angiography, which confirmed the mycotic aneurysm.

Our patient's blood culture result was negative. It is important to consider that 2 to 7% of all pediatric IE cases have negative blood cultures, similar to adult cases.[15,16] However, to be declared "blood culture-negative infective endocarditis," the patient must meet the condition of negative cultures from inoculation of at least three independent blood samples in a standard blood culture system, after five days of incubation and subculturing.[17,18] In IE with negative cultures, one must consider an indolent organism or a fungal cause.[17] However, since our patient improved as shown by the reductions in vegetation sizes, it was unlikely that such atypical organisms were the cause. A limitation of this conclusion is that we made only a single attempt to culture blood from our patients.

Ceftriaxone and gentamicin were chosen as the empiric antimicrobial regimen for our patients. Ceftriaxone has good coverage of both Gram-positive and negative organisms; gentamicin covers mostly Gram-negative organisms.[10,11] Most recent IE infections are caused by methicillin-resistant Staphylococcus aureus, with the incidence of MSSA in IE on the rise.[19] The infective endocarditis in our patients did not require surgery. Closure of the VSD was planned after recovery from endocarditis. Post-endocarditis evaluation of the valves and VSD anatomy by echocardiography will be done before deciding on VSD closure by surgery or occlusion.

We acknowledge that current recommendations regarding empirical antimicrobial regiment in treating infective endocarditis mandate the use of vancomycin and gentamycin as the initial empirical antimicrobial agents of choice. However, difficulty in procuring vancomycin resulted in ceftriaxone being chosen as an empirical agent in this case. We suspected that the presence of congenital heart malformation in our patient may have been the inciting factor for the presumed bacterial infection.

The reported IE incidence rates in the children with congenital heart disease range from 40 to 60 per 100,000 person-years, which is several times higher than in general pediatric populations.[20] In these studies, ventricular septal defects put patients at higher risk for developing infective endocarditis, as seen in our patient. Other risk factors include cardiac surgery within six months and age <3 years.[20,21]

In conclusion, hemorrhagic stroke is a rare, initial presentation of pediatric infective endocarditis. Neurologic involvement, such as mycotic aneurysm and hemorrhage, should be considered as a presenting manifestation of infective endocarditis, irrespective of cardiac malformation. Early recognition and intervention will hopefully decrease morbidity. A broad but appropriate range of differential diagnoses should be considered in pediatric patients who present with stroke.

Conflict of Interest

None declared.

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Original Article

Risk factors for sleep problems in infants

Hesti Lestari, Audrey Mety Iriani Wahani, Rocky Wilar, Permatami Herwansyah

Abstract

Background Sleep disorders in infants can cause developmental problems, suboptimal growth, behavioral disorders, fatigue, irritability, impulsiveness, and poor mother-infant bonding.

Objective To evaluate possible risk factors for sleep disorders in infants.

Methods This cross-sectional study was conducted in healthy infants aged 3-6 months. Subjects were selected using proportional random sampling from four different primary healthcare facilities in Manado, North Sulawesi. Their parents completed the Brief Infant Sleep Questionnaire. Sleep disorder was defined as the presence of one or more conditions including sleep duration less than 9 hours at night (from 19.00 until 07.00), waking up at night (from 22.00 until 06.00) more than 3 times, and more than 1 hour waking at night.

Results Of 112 subjects, 58 (51.8%) were male. Subjects' mean age was 4.21 (SD 0.829) months and 76 (67.86%) experienced sleep disorders. Sleep disorders had significant associations with low socioeconomic status (OR 17; 95%CI 3.8 to 75.8), middle school or lower maternal education (OR 44.5; 95%CI 9.8 to 202), non-supine sleeping position (OR 8.8; 95%CI 1.9 to 39.7), parental use of electronic devices (OR 156.2; 95%CI 35.1 to 692.9), and non-exclusive breastfeeding (OR 85.2; 95%CI 21.1 to 344.2). Correlative analyses also revealed that electronic media usage had the strongest association with sleep disorders, followed by breastfeeding pattern, maternal education, socioeconomic status, and sleeping position (0.839, 0.771, 0.624, 0.433, and 0.309, respectively). However, there were no significant correlations upon multivariate analysis.

Conclusion Parental use of electronic media before sleeping is the strongest risk factor for sleep disorders among infants, followed by non-exclusive breastfeeding pattern, low maternal education, low socioeconomic status, and non-supine sleeping position. However, none of these correlations were significant upon multivariate analysis, this show that all these factors influence sleep together. **[Paediatr Indones. 2020;60:186-91; DOI: 10.14238/pi60.4.2020.186-91]**.

Keywords: *infants; sleep; questionnaires; media*

Sleep is a condition of regular, reversible, and rapid rest characterized by reduced body movement and decreased awareness of the surroundings.[1] Sleep is essential, particularly in infants.[1-3] Sleep disorders are a series of symptoms marked by disturbances in the quantity, quality, and sleep duration.[4] Many children suffer from inadequate sleep, both in terms of quality and quantity.[5-7] A study found a 44.2% prevalence of sleep disorders in children under three years of age in five cities in Indonesia.[8] Sleep disorder in infants can cause developmental problems, suboptimal growth, behavioral disorders, fatigue, irritability, impulsiveness, and can affect the relationship between a mother and her baby.[9]

Sleep disorders in infants are influenced by several factors, both internal and external. Internal factors are infants' characteristics, such as gender, history of low APGAR scores, prematurity, various neuropsychiatric disorders, and chronic diseases. External factors that can affect sleep include sleeping position, parental use of electronic devices

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and media before sleeping, not breastfeeding, as well as family and environmental conditions such as socioeconomic status, educational status, and parental characteristics.[10-12] *The Brief Infant Sleep Questionnaire* (BISQ) is a well-validated instrument for capturing sleep disorders in infants up to 3 years of age.[13,14] This instrument consists of several indicators of sleep quality. A previous study reported that BISQ can be used as a screening tool for sleep disorders in infants.[14] As such, we aimed to assess for risk factors for sleep disorders in infants using BISQ.

Methods

The study was conducted at Minanga, Sario, Teling Atas, and Wawonasa primary healthcare, which were randomly selected from a total of 13 primary healthcare facilities serving in Manado City, North Sulawesi Province. The inclusion criteria were healthy infants aged 3-6 months. Infants with a history of low birth weight or prematurity were excluded. The minimum required sample size was calculated using the 'rule of thumb' formula, i.e., the recommended number of subjects was 20 times the total number of independent variables to be examined. To anticipate some subjects' dropping out, the minimum required sample size was 112 subjects.

Sample collection was conducted using proportional random selection of the eligible subjects from 522 infants aged 3-6 months at the four previously selected primary healthcare centers, resulting in a total of 112 subjects. All parents/guardians of eligible infants were given explanations of the benefits and objectives of the study. If the parents/guardians agreed to participate, they were asked to sign an informed consent form. Healthy infants were considered to be babies who were in good health, free from disease conditions, able to participate in activities without any physical limitations. For our study, infants' health conditions were determined by their clinical history and physical examinations. Sleep disorders were determined by the *Brief Infant Sleep Questionnaire* (BISQ) filled by parents, the results of which were broad categories of yes and no.

Sleep disorder was defined as the presence of one or more conditions including sleep duration at night (from 19:00 until 07:00) <9 hours, waking up at night (from 22:00 until 06:00) more than 3 times, and >1 hour of being awake at night. We aimed to investigate factors that influence sleep disorders in infants, including parental use of electronic media within one hour before going to bed in one room with their infant, socioeconomic status, maternal education, breastfeeding pattern, and sleeping position. Electronic use was defined as electronic tools that send and receive information using electronics, such as televisions, computers, video games, mobile phones, internet and others. Socioeconomic status in this study was based on the provincial minimum wage of North Sulawesi Province. Low socioeconomic status was defined as income less than 2,824,286 IDR/month. Maternal education was defined as mother's final education level and divided into high school graduate and middle school/lower graduate. Breastfeeding pattern was divided into exclusive and non-exclusive. Exclusive breastfeeding was defined as receiving breast milk only without the addition of other liquid or solid food. Sleeping position was the most frequent sleeping position and divided into supine and non-supine. During questionnaire assessment (BISQ), parents were interviewed by one of researchers.

Univariate analysis was performed to analyze the characteristics of subjects, while bivariate analysis was performed to analyze for possible correlations between each variable and sleep disorders [Chi-square test and correlation coefficient phi ($r\phi$)]. The most influential factor was shown by the magnitude of the correlation value ($r\phi$). Higher values indicated stronger associations as follows: 0=no correlation between two variables, >0 - 0.25=very weak correlation, >0.25 - 0.5=moderate correlation, >0.5 – 0.75=strong correlation, >0.75 - 0.99=very strong correlation, and 1=perfect correlation.

Significant variables were further analyzed by calculating odds ratios (OR) with 95% confidence intervals (CI). Multivariate analysis was carried out to collectively analyze the independent variables to determine which factors contributed the most to the occurrence of sleep disorders. Results with $P<0.05$ were considered to be statistically significant. Data were analyzed using *SPSS version 25*. This study was approved by the Ethics Committee of Prof. Dr. R. D. Kandou Hospital, Manado.

Results

The characteristics of the 112 study subjects are shown in **Table 1**. Chi-square analysis revealed that low socioeconomic status was a significant risk factor for sleep disorders (OR 17.0; 95%CI 3.8 to 75.8; P<0.0001), as was middle school or lower maternal education (OR 44.5; 95%CI 9.8 to 202; P <0.0001). A non-supine sleeping position had an 8.8 times higher risk of sleep disorders (OR 8.8; 95%CI 1.9 to 39.7; P <0.0001). In addition, parental use of electronic media within one hour before going to bed in one room with infants (OR 156.2; 95%CI 35.1 to 692.9; P <0.0001) and non-exclusive breastfeeding (OR 85.2; 95%CI 21.1 to 344.2; P<0.0001) were significant risk factors for sleep disorders (**Table 2**).

Correlation coefficient analysis values ( )revealed that parental use of electronic media had the strongest positive association with sleep disorders, followed by non-exclusive breastfeeding, middle school or lower maternal education, low socioeconomic status, and supine sleeping position (**Table 3**).

Multivariate analysis with logistic regression of the five variables revealed no significant associations between sleep disorders and socioeconomic status, maternal education level, sleep position, parental use of electronic media, and breastfeeding pattern (P=0.995, P=0.997, P=0.998, P=0.995, and P=0.993, respectively).

Table 1 . Characteristics of subjects

Characteristics	(N=112)
Mean age (SD), months	4.21 (0.83)
Gender, n (%)	
Female	54 (48.2)
Male	58 (51.8)
Maternal occupational status, n (%)	
Working	33 (29.5)
Not working	79 (70.5)
Socioeconomic status, n (%)	
Low	40 (35.7)
Middle-high	72 (64.3)
Maternal education, n (%)	
High school graduate	55 (49.1)
Middle school/lower graduate	57 (50.9)
Sleeping position, n (%)	
Supine	84 (75)
Non-supine	28 (25)
Electronic media use, n (%)	
Yes	74 (66.1)
No	38 (33.9)
Breastfeeding pattern, n (%)	
Exclusive	31 (27.7)
Not exclusive	81 (72.3)
Sleep disorders, n (%)	
Yes	76 (67.9)
No	36 (32.1)

Table 3. Correlation coefficient ($r\phi$) of factors attributed to sleep disorders

Risk factors	Correlation coefficient phi ($r\phi$)	P value
Parental electronic media use	0.839	< 0.0001
Breastfeeding pattern	0.771	< 0.0001
Maternal education	0.624	< 0.0001
Socioeconomic status	0.433	< 0.0001
Sleeping position	0.309	0.001

Table 2. Associations between various risk factors and sleep disorders in infants

Risk factors	Sleep disorder, n(%)	No sleep disorder, n(%)	Total, n(%)	Odds ratio (95%CI)	P value
Socioeconomic status					
Low	38 (95)	2 (5)	40 (100)	17 (3.8 to 75.8)	<0.0001
Middle-high	38 (52.8)	34 (47.2)	72 (100)		
Maternal education					
Middle school or lower	55 (96.5)	2 (3.5)	57 (100)	44.5 (9.8 to 202.0)	<0.0001
High school	21 (38.2)	34 (61.8)	55 (100)		
Sleeping position					
Non-Supine	26 (92.8)	2 (7.2)	28 (100)	8.8 (1.9 to 39.7)	<0.0001
Supine	50 (59.5)	34 (40.5)	84 (100)		
Parental electronic media use					
Yes	71 (95.9)	3 (4.1)	74 (100)	156.2 (35.1 to 692.9)	<0.0001
No	5 (13.2)	33 (86.8)	38 (100)		
Breastfeeding pattern					
Not exclusive	73 (90.1)	8 (9.9)	81 (100)	85.2 (21.1 to 344.2)	<0.0001
Exclusive	3 (9.7)	28 (90.3)	31 (100)		

Discussion

Sleep disorders in children are characterized by a disturbance in the quantity, quality, or sleep time.[15] In the first year of life, a child's sleep patterns undergo dramatic changes. The maturation process of the circadian rhythm occurs at the age of 2-3 months, accompanied by a change in the sleep patterns. In particular, increased sensitivity to light underlies the gradual transition from polyphasic sleep patterns during the neonatal period to nighttime sleep patterns and being awake during the day.[16]

We evaluated for possible correlations between sleep disorders and socioeconomic status, maternal education, sleeping position, parental use of electronic media, and breastfeeding patterns. The BISQ evaluation revealed that 76 of 112 infants (67.86%) had sleep disorders. This finding was in agreement with a previous study where Asians infants and toddlers tend to experience sleep disorders compared to Caucasians with a prevalence of 51.90% and 26.30%, respectively.[17]

Low socioeconomic status has been associated with significant limitations in the quality of family life, such as nutritional status, health, and education. Children from low income families have higher rates of breathing problems during sleep, shorter sleep time, poorer sleep quality, and increased nap time.[11] In our study, infants with low socioeconomic status had 17 times higher risk of developing sleep disorders in the bivariate analysis. Similarly, a previous study reported that environmental, medical, and psychosocial factors were associated with sleep.[7] With regards to the housing environment, families with lower socioeconomic status tend to have fewer bedrooms and more people sharing one room. Ventilation systems may be far from ideal, as high levels of allergens can affect the children's sleep quantity and quality.[18]

Maternal education level may affect the way mothers take care of their children. As parenting patterns are closely related to child development, higher parental education may lead to more informed and better care of children, thus positively affecting child development. Conversely, lower maternal education could negatively impact parenting patterns, hampering child development. In our study, lower maternal education had 44 times higher risk of developing sleep disorders. A study reported that family influence on children's sleep patterns mainly centers on the attitudes and behavior of parents towards their children.[11] Responsive and interactive parental attitudes towards children and a conducive home environment can reduce sleep disorders in children. Specifically, regarding the lack of mental stimulation by parents, it is generally attributed to the low level of education they have so that they do not understand how important quality and quantity of sleep are correlated to a child's growth and development.[11]

In our study, infants with non-supine sleeping positions had an 8.8 times higher risk for developing sleep disorders. A previous study reported that babies who slept in a prone position had lower metabolic rates compared to those who slept in a supine position. Although heat production decreases, on prone position, the body surface temperature would otherwise increase due to heat transfer from central to peripheral body parts. Skin vasodilation results in a decrease in the central-to-peripheral temperature gradient and an increase in heart rate. In the prone sleeping position, increased heart rate, respiratory rate, and respiratory quotient (RQ) have been noted.[19]

Rapid technological advancement and robust exposure to electronic media have increased our awareness and knowledge, however, they are risk factors for irregular sleep habits, shorter sleep duration, and sleep disorders, especially in older child and youth. These associations were primarily due to time spent on portable devices such as phones or tablets, rather than stationary devices such as TV or video game consoles.[20] A study noted that exposure to bright screens from electronic media is sufficient to inhibit melatonin production.[21] On the other hand, room lights can also inhibit melatonin production when watching television in a bright room. Another study showed that electronic media exposure including access to media devices in the bedroom, although not actively used before bedtime, was associated with sleep disturbances disorders in children aged 6 to 19 years (OR 1.79; 95% CI 1.39 to 2.31).[22] In our study, we found parental electronic use within one hour before going to bed with infants had an 152 times higher risk for developing sleep disorders. Although the mechanism of the association between parental media use and sleep disorders in infants

is unknown, studies in older children have been extensively investigated. Whether the association was through melatonin inhibition as shown in older children, still need further studies.[20]

Exclusive breastfeeding means providing breast milk without any additional foods and drinks. Exclusive breastfeeding for the first six months of life is recommended. Melatonin is an important component of breast milk. It is secreted at night in adults, but not in infants. A previous study reported that breast milk contains melatonin in a circadian fashion, similar to levels of melatonin in blood and saliva. In human milk, melatonin levels increase at night, reaching a peak at around 3:00 in the morning, followed by undetectable levels throughout the day. Benefits of melatonin was not found in formula-fed infants. Melatonin exerts some beneficial effects in improving sleep quality of infants and can reduce infantile colic.[23] In our study, infants with non-exclusive breastfeeding had an 85 times higher risk for developing sleep disorders.

The $r\phi$ value is used to compare the influence of various risk factors. Our $r\phi$ analysis revealed that parental use of electronic media, breastfeeding patterns, maternal education, socioeconomic status, and sleeping position demonstrated robust correlations with sleep disorders ($r\phi$ values of 0.839, 0.771, 0.624, 0.433, and 0.309, respectively. However, multivariate logistic regression test revealed that none of the risk factors were significantly associated with sleep problems ($P > 0.05$). This is because each of the risk factors in this study influences the occurrence of sleep problems in infants.

The strengths of this study were that it was the first study in Indonesia regarding risk factors for sleep disorders in infants, especially for the use of electronic media in the bedroom, as well using proportional random sampling. Limitations of this study were not evaluating several factors that could influence sleep disorders, such as maternal medical condition, nutritional status, and parenting. In our study, we did not explore further type of electronic media, which in other study has found differences.[20] In addition, this study was cross-sectional, hence, the onset of sleep disorders could not be evaluated.

In conclusion, there are strong correlations between low socioeconomic status, low maternal education, supine infant sleep position, parental use of electronic media, and non-exclusive breastfeeding with sleep disorders. The use of electronic media by parents in the same room as the infant before going to sleep is the strongest risk factor associated with sleep disorders, followed by breastfeeding pattern, maternal education, socioeconomic status, and sleep position. However, none of the above correlations are significant by multivariate analysis, means that each of the risk factors influences the occurrence of sleep disorders in infants.

Conflict of Interest

None declared.

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Original Article

Validity and reliability update of the *Indonesian version of International Society for Prevention of Child Abuse and Neglect - Child Abuse Screening Tool* (ICAST-C)

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Abstract

Background In Indonesia, few screening tools for child abuse and neglect are available. The currently-favored tool was adapted from the *International Society for Prevention of Child Abuse and Neglect* (ISPCAN)-*Child Abuse Screening Tool* (ICAST-C) questionnaire, consisted of 5 domains child abuse and 59 items.

Objective To re-evaluate the validity and reliability of the Indonesian version of ICAST-C.

Methods A cross-sectional study was conducted on 480 children aged 11-18 years from junior and senior high schools in Bandung, West Java, Indonesia. Subjects were selected using two-stage cluster sampling. A validity test using Spearman's rank correlation with Rs ≥0.3 was considered valid. A reliability test using Cronbach's alpha formula with alpha score ≥0.7 was considered reliable.

Results Most items in the Indonesian version of ICAST-C were valid and reliable, except for the following 9 out of 59 items: "anyone in your home used alcohol", "seen adults in your home use knives", "insulted you by calling you dumb", "in order stop or change behavior", "forbade you from going out", "pinched you", "explained to you why something you did was wrong", "gave you something else to do (in order to stop or change behavior)", and "took away privileges or money". The ICAST-C reliability was good (0.919), however domain of violence exposure (0.483) and neglect (0.445) were not so good.

Conclusion The updated Indonesian version of ICAST-C is considered valid and reliable as a screening tool for child abuse. **[Paediatr Indones. 2020;60:218-23; DOI: 10.14238/pi60.4.2020.218-23]**.

Keywords: *child abuse; neglect; screening tool; Indonesian version of ICAST-C*

Child abuse or maltreatment or violence against children (VAC) is any kind of physical and mental violence, sexual abuse, neglect or negligent treatment, and commercial or other exploitation, that has high likelihood of resulting in actual or potential harm to child health, survival, development, dignity, responsibility, belief, or rights.[1-4] According to the *World Health Organization* (WHO), 25-50% of children are victims of physical violence, while 20% of girls and 5-10% of boys are victims of sexual abuse.[5]

Indonesian Ministry of Health reports incidence of violence against children increases each year. Most of violence against children cases was found in health facilities and/or police departments. The iceberg phenomen is considered since a lot of unreported cases. The Indonesian culture or noms may caused of low of detection rate of violence against children. Moreover case was founded on severe violence or

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even death.[2] Screening tools to detect violence against children established and applied in Indonesia include the ICAST-C (Indonesian version), *Parent-Child Conflict Tactics Scale, Adverse Childhood Experiences Study Questionnaire* (ACEs), and the *Lifetime Victimization Screening Questionnaire*.[1,6-8] ICAST-C is generally accepted as the best, since it includes five violence domains (violence exposure, physical, psychological, sexual, and neglect).[8,9]

The Indonesian version of ICAST-C has followed five translation phases, cultural adaptation, and initial validity and reliability testing.[6] The original ICAST-C was used to collect information on neighborhood violence. It has been analyzed by more than 130 experts from 43 countries and adapted into 20 languages, with valid and reliable results using Cronbach's alpha analysis.[9-11] This study was done to evaluate the validity and reliability of the Indonesian version of ICAST-C. The questionnaire passed the translation and cross-cultural adaptation phases before being applied in Indonesia.[12-14] The results of this study can potentially be used to improve the validity and reliability of the tool by updating question phrasing and order.

Methods

This cross-sectional study was done on junior and senior high school students aged 11-18 years in Bandung, West Java, Indonesia, from August to November 2016. Subjects were included using two-stage cluster sampling. First, several schools were selected (2 primary and 2 secondary high schools) then the adequate number of students was determined by a simple random sampling method. The minimum required sample size was 255 subjects based on the formula:

$$n = \left[\frac{Z_\alpha + Z_\beta}{0.5 \ln(1+r)/(1-r)} \right]^2 + 3$$

Z_α = alpha standard deviation (1.96)
Z_β = beta standard deviation (1.64)
r = minimal significant correlation (0.5)

Children who refused to join the study or were unavailable to return the informed consent form were excluded. We used Indonesian version of ICAST-C questionnaire, which was a self-report questionnaire, consisted of 5 child abuse domains and 59 items (7 violence exposure, 18 physical, 19 psychological, 4 sexual, and 11 neglect). Subject filled the ICAST-C questionnaire anonymously in the classroom, supervised by the teacher and assistant investigator. Subject who were unable to completely fill the questionnaire were considered to be dropped out of the study.

The item validity of ICAST-C was assessed by Spearman's rank correlation. Reliability or internal consistency was assessed by Cronbach's alpha analysis. Spearman's rank correlation coefficient was considered to be valid for rs ≥0.3, while internal consistency was considered to be reliable for Cronbach's alpha ≥0.7. Data processing was performed with *SPSS software version 21.0*. This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran.

Results

A total of 500 consent forms were distributed to students at the chosen schools, 480 of whom were included in the study. A total of 20 students were excluded due to not returning the signed parental informed consent form (9 students) and refusing to join the study (11 students). Questionnaires were filled anonymously and all had complete data.

Subjects' characteristics data were included sex, age, parental education, number of family members, neighborhood, and race as presented in **Table 1**. Subjects' ages ranged from 11 to 18 years with a mean of 14.87 (SD 1.80) years. Parental education varied from elementary school to post-graduate, with an average educational level of senior high school (36.5% of fathers and 40.4% of mothers). Most subjects had small families (≤4 family members) (93.8%), lived with their parents (85.8%), and were Sundanese (72.9%).

The item validity of violence exposure domain, resulting 2 out of 7 items were not valid. Those items were exposure to "anyone in your home used alcohol" and "seen adults in your home use knives" (**Table 2**). The item validity of psychological violence domain revealed, 3 out of 19 items were not valid: "insulted

Table 1. Subjects' characteristics

Characteristics	N=480
Gender, n (%)	
Male	232 (48.3)
Female	248 (51.7)
Age, years	
Mean (SD)	14.87 (1.80)
Median (range)	15.00 (11.00-18.00)
Education, n (%)	
Paternal	
Elementary	40 (8.3)
Junior high	34 (7.1)
Senior high	175 (36.5)
Diploma	33 (6.9)
Bachelor's	13 (2.7)
Magister-doctoral degree	135 (28.1)
Not known	50 (10.4)
Maternal	
Elementary school	54 (11.3)
Junior High school	42 (8.8)
Senior high school	194 (40.4)
Diploma	42 (8.8)
Bachelor	106 (22.1)
Magister-doctoral degree	8 (1.7)
Not known	34 (7.1)
Birth position in the family, n (%)	
First born	170 (35.4)
Middle	116 (24.2)
Last	165 (34.4)
Single	29 (6.0)
Number of family members, n (%)	
≤4	450 (93.8)
>4	30 (6.3)
Living with, n (%)	
Parents	412 (85.8)
Relatives	59 (12.3)
Dormitory/orphanage	9 (1.9)
Ethnicity, n (%)	
Sundanese	350 (72.9)
Javanese	37 (7.7)
Sumateranese	12 (2.5)
Betawinese	3 (0.6)
Mixed	78 (16.3)

you by calling you dumb", " gave you something else to do (in order to stop or change behavior)," and "forbade you from going out" (**Table 3**). Of the 18 item of physical violence domain, only "pinched you" was considered invalid (**Table 4**). Regarding on neglect domain, 3 of 11 items were not valid: "explained to you why something you did was wrong," "gave you a reward for behaving well" and "took away privileges or money" (**Table 5**). In addition, all 4 variables in the sexual abuse domain were considered valid (**Table 6**).

The reliability test was assessed by internal consistency of one session analysis. A group of items was considered reliable and success in analyzing the item if the reliability coefficient was ≥0.7. The Cronbach's alpha results are presented in **Table 7**. The variables had alpha values of 0.483 to 0.966. The violence exposure as well as negligence domains had alpha values of 0.483 and 0.445, respectively, so they were interpreted as unreliable. The total alpha value of 59 items was 0.919, hence, the Indonesian version of ICAST-C was considered to be reliable and valid.

Table 2. Item validity test for violence exposure

No	Variables	Rs	Conclusion
1	Has anyone in your home used alcohol (q1.12)	0.179	Invalid
2	Shouting and screaming (q1.13)	0.485	Valid
3	Hurting each other (q1.14)	0.482	Valid
4	Seen adults in your home use knives (q1.15)	0.271	Invalid
5	Anyone close to you has been murdered (q4.1)	0.413	Valid
6	Living with violence (q4.2)	0.692	Valid
7	Has anyone come into your home and stolen something (q4.3)	0.689	Valid

Table 3. Item validity test for psychological violence domain

No	Variables	Rs	Conclusion
1	Tried to embarrass you because you (q1.16)	0.178	Invalid
2	Shouted, yelled, or screamed at you very loudly (q2.1)	0.438	Valid
3	Insulted you by calling you dumb, lazy (q2.2)	0.476	Valid
4	Cursed you (q2.3)	0.632	Valid
5	Blamed you for his/her misfortune (q2.5)	0.602	Valid
6	Told to stop or start doing something (q2.6)	0.326	Valid
7	Told to change behavior (q2.9)	-0.098	Invalid
8	Forbade you from going out (q2.11)	0.196	Invalid
9	Embarrassed publicly (q2.12)	0.562	Valid
10	Said wish you were dead or never been born (q2.13)	0.551	Valid
11	Threatened to leave or abandon (q2.14)	0.646	Valid
12	Locked out (q2.15)	0.613	Valid
13	Threatened with words (q2.16)	0.609	Valid
14	Threatened to hurt or kill (q2.17)	0.576	Valid
15	Referred to you skin color/gender/ religious (q2.37)	0.537	Valid
16	Embarrassed because orphan (q2.38)	0.515	Valid
17	Stopped you from being with others (q2.39)	0.572	Valid
18	Stole/broke your belongings (q2.40)	0.476	Valid
19	Threatened with bad marks that were undeserved (q2.41)	0.607	Valid

Table 4. Item validity test for physical violence domain

No	Variables	Rs	Conclusion
1	Kicked (q2.18)	0.607	Valid
2	Shook aggressively (q2.19)	0.499	Valid
3	Slapped on the face or back of head (q2.20)	0.655	Valid
4	Hit on the head with knuckles (q2.21)	0.693	Valid
5	Spanked on the bottom with bare hand (q2.22)	0.501	Valid
6	Hit on the buttocks with an object (q2.23)	0.701	Valid
7	Hit elsewhere (not buttocks) with an object (q2.24)	0.664	Valid
8	Hit over and over (q2.25)	0.669	Valid
9	Choked (q2.26)	0.652	Valid
10	Burned, scalded, or branded q2.27)	0.595	Valid
11	Put hot pepper, soap or spicy food in your mouth (q2.28)	0.615	Valid
12	Locked you up or tied you to restrict (q2.29)	0.656	Valid
13	Twisted your ear (q2.30)	0.354	Valid
14	Pulled hair (q2.31)	0.560	Valid
15	Pinched you(q2.32)	0.181	Invalid
16	Force to stand, sit, or kneel and causing pain (q2.33)	0.673	Valid
17	Put in time-out (q2.34)	0.622	Valid
18	Given drugs or alcohol (q2.36)	0.640	Valid

Table 5. Item validity test for neglect domain

No	Variables	Rs	Conclusion
1	You did not get enough to eat and or drink (q3.1)	0.575	Valid
2	You had to wear clothes that were dirty, torn, or inappropriate (q3.2)	0.467	Valid
3	You were not taken care of when you were sick/injured (q2.3)	0.600	Valid
4	Lack of supervision (q2.4)	0.597	Valid
5	Feeling uncared for (q2.5)	0.654	Valid
6	Feeling unimportant (q2.6)	0.719	Valid
7	Ignored (q2.4)	0.484	Valid
8	Explained to you why something you did was wrong (q2.7)	-0.058	Invalid
9	Gave you a reward for behaving well (q2.8)	-0.010	Invalid
10	Took away privileges or money (q2.10)	-0.019	Invalid
11	Withhold meal as punishment (q2.35)	0.473	Valid

Table 6. Item validity test for sexual abuse domain

No	Variables	Rs	Conclusion
1	Made to look private parts	0.947	Valid
2	Made to touch private parts	0.950	Valid
3	Made a sex video or took photographs	0.931	Valid
4	Forced to have sex	0.933	Valid

Table 7. Internal consistency (Cronbach's alpha) of the 5 domains

Domain	Violence exposure	Psychological violence	Physical violence	Neglect	Sexual abuse
# Number of item	7	19	18	11	4
Alpha	0.483	0.805	0.879	0.445	0.966

Discussion

The International Society for Prevention of Child Abuse and Neglect (ISPCAN)*-Child Abuse Screening Tool* (ICAST-C) to detect violence against children was developed by the United Nations Secretary-General. It was designed for application in cross-cultural and multinational research on violence against children in various countries and times.[11]

The first study of the Indonesian version of ICAST-C showed that it had good validity and reliability (Kuder-Richardson score 0.919).[6] In our study with 480 junior and senior high students aged 11 to 18 years in Bandung, the validity of items for 5 domains of violence against children was analyzed by Spearman's rank correlation coefficient. Our subjects were from different social classes or social groups. As such, subjects varied by income, class, race, ethnicity, and/or dialect to ensure that we catch challenges to completion of the questionnaire unique to those factors. Since subjects completed the questionnaires in group settings, they were prohibited from asking other subjects how they answered questions or from seeing how others answered specific questions.

We found the ICAST-C Indonesian version to be valid except for 9 of 59 variables. Those items were "has anyone in your home used alcohol (q1.12),"seen adults in your home use knives", "tried to embarrass you because you", "instructed to change behavior", "forbade you from going out", "pinched you", "explained to you why something you did was wrong", "gave you a reward for behaving well", and "took

away privileges or money". A study of the Brazilian-Portugese adaptation of ICAST-C noted that 7 of 59 variables were not valid and had to be changed or eliminated.[15]

A previous study showed reliable alpha values for three ICAST instrument ranging from 0.69 to 0.89.[11] However, in our study the total alpha value was 0.919, which was considered to be very good reliability, despite the unreliable alpha values for domains of violence exposure and neglect (0.483 and 0.445, respectively). Another study also reported a less reliable alpha value (0.69) for the violence exposure domain.[10]

To our knowledge, the Indonesian version of ICAST-C has not been compared to other instruments, thus, there is no available data on criterion-related validity, sensitivity, and specificity. The limitation of this study was the lack of test-retest reliability and construction and criterion-related validity of the Indonesian version of ICAST-C compared to other questionnaires. In addition, the Indonesian version of ICAST-C does not include the frequency and classification of violence type, which makes severity assessment challenging. A suggestion for further study is to include the frequency of violence and classification of violence (severe or mild). We believe it would be helpful for the reporting system and management of violence against children.

In conclusion, the Indonesian version of ICAST-C still needs improvement on 9 of 59 invalid items. Further study is needed to analyze criterion-related validity, sensitivity, and specificity of ICAST-C compared to other screening tools.

Conflict of Interest

None declared.

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Original Article

Blood glucose level during induction phase chemotherapy in childhood acute lymphoblastic leukemia

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Abstract

Background Steroids and L-asparaginase (L-Asp) are agents used in induction phase chemotherapy for childhood acute lymphoblastic leukemia (ALL). Both agents are often reported to have the side effect of hyperglycemia, and native L-Asp is also reported to cause hypoglycemia. In ALL patients, hyperglycemic events during chemotherapy can cause lower 5-year overall and relapse-free survival.

Objective To investigate the incidence of abnormal blood glucose level (BG) as the side effect of prednisone and L-Asp during induction phase chemotherapy, its predisposing factors, and its effect on remission status.

Methods This cohort prospective study was conducted in 36 children aged 1-18 years who were newly diagnosed with childhood ALL at Dr. Moewardi Hospital, Surakarta, Central Java. Subjects' nutritional status consist of wellnourished and undernourishment. Subjects underwent BG monitoring. At the end of induction phase chemotherapy, subjects underwent bone marrow puncture (BMP) evaluation to assess their response to chemotherapy and the effect of abnormal BG on remission status.

Results Hypoglycemia, a combination of hypoglycemia and hyperglycemia, hyperglycemia, as well as euglycemia, were experienced by 9, 7, 6, and 14 subjects, respectively. Nutritional status was found to be a significant risk factor for abnormal BG. There was no significant difference in remission status at the end of induction phase chemotherapy between the euglycemic group and abnormal BG groups (P=0.533).

Conclusion Abnormal BG during induction phase chemotherapy did not affect remission status at the end of induction phase. Undernourishment is also found to be a predisposing factor in abnormal BG. **[Paediatr Indones. 2020;60:192-7; DOI: 10.14238/pi60.4.2020.192-7]**.

Keywords: *blood glucose; hyperglycemia; hypoglycemia; L-asparaginase; steroids; leukemia*

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy.[1,2] Chemotherapy is still the main therapy for childhood ALL. Steroids and L-Asp are agents used in inductive phase chemotherapy known to have hyperglycemia as a side effect.[3-5] A previous study reported that hyperglycemia during inductive phase chemotherapy was associated with lower 5-year overall and relapse-free survival.[6] Native L-Asp, produces more incidences of hypoglycemia than hyperglycemia during chemotherapy for childhood ALL.[7] To our knowledge, there have been no studies on the impact of hypoglycemia during chemotherapy on survival rate. As such, we aimed to investigate the incidence of abnormal blood glucose level (BG) as the side effect of prednisone and L-Asp used in inductive phase chemotherapy, predisposing factors of abnormal BG, and the effect of abnormal BG on remission status.

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Methods

This prospective cohort study was conducted to analyze the effect of abnormal BG caused by prednisone and L-Asp treatment on remission status in subjects treated in the Pediatric Hemato-Oncology Ward, Dr. Moewardi General Hospital, Surakarta, Central Java, Indonesia. The study was performed between June 2018 and June 2019. Subjects were pediatric patients newly diagnosed with ALL, aged between 1 and 18 years, who underwent chemotherapy following the Indonesia ALL 2018 high risk or standard risk protocol.8 All the participants' parents or caregivers provided written informed consent prior to this study. Patients with abnormal BG before chemotherapy, HbA1C ≥ 6.5%, or incomplete induction phase were excluded from the study. The ALL diagnoses were established with bone marrow puncture (BMP) examinations. Patients were followed from the time of established diagnosis until BMP evaluations at the end of induction phase chemotherapy, a total period of 8 weeks. For baseline, HbA1C measurements as well as fasting and two-hour postprandial BG examinations were done before the chemotherapy started. In addition, fasting and two-hour postprandial BG evaluations were done at the end of each week during induction phase chemotherapy. In particular weeks when L-Asp was added for chemotherapy, BG examinations were done after the second dose. The baseline characteristics of subjects were sex, age, risk stratifications, and nutritional status. At the end of inductive phase, all subjects were evaluated with BMP examinations. Complete remission was achieved when blast cells were <5%, incomplete remission was defined as blast cells 5% - 20%, and no remission was defined as blast cells >20% of the 200 nucleated cells in bone marrow. Incomplete remission was categorized as no remission.

Blood glucose of <65 mg/dL and 65-199 mg/dL were defined as hypoglycemia and euglycemia, respectively, while hyperglycemia was described as BG ≥126 mg/dL for fasting or ≥200 mg/dL for two-hour postprandial exams.9,10 The subjects were divided in two groups, euglycemia (normal BG during induction phase chemotherapy) and abnormal. The abnormal group was sub-divided into three subgroups: hypoglycemia (≥ 1 episode of hypoglycemia), combination hypoglycemia and hyperglycemia (≥ 1 episode of hypoglycemia and ≥ 1 episode of hyperglycemia), or hyperglycemia (≥ 1 episode of hyperglycemia) during induction phase chemotherapy.

We analyzed for possible associations between abnormal BG and sex, age, risk stratification, and nutritional status. Age was divided into <10 years and ≥10 years. Risk stratifications were classified into standard risk and high risk, based on the Indonesia ALL 2018 protocol. Nutritional status was assessed with weight per height measurements and categorized based on Waterlow 1972.11 Data were analyzed with SPSS version 21.0. Bivariate analysis was done by Chi-square test and was followed by multivariate logistic regression analysis. This study was approved by the Health Research Ethics Committee of Universitas Sebelas Maret Medical School/Dr. Moewardi Hospital Surakarta.

Results

Forty-five children were diagnosed with ALL during study period, but two were excluded due to hyperglycemia before chemotherapy. The remaining 43 patients were included, but 7 died before completing the induction phase, hence, 36 subjects completed the study. The subjects' baseline characteristics are presented in **Table 1**. Half of the subjects were female. Twenty-four subjects were under 10 years of age, 25 subjects were in the high risk stratification category, and 20 subjects were well-nourished (**Table 1**).

Blood glucose exams during induction phase demonstrated hyperglycemia in 6 subjects, hypoglycemia in 9 subjects, hypoglycemia and hyperglycemia in

Table 1. Baseline characteristics of subjects

Characteristics	(N=36)
Gender, n	
Male	18
Female	18
Age, n (%)	
<10 years	24
≥10 years	12
Risk stratification, n	
High risk	25
Standard risk	11
Nutritional status, n	
Well-nourished	20
Undernourished	16

7 subjects, and euglycemia in 14 subjects. The onset of abnormal BG is shown in **Table 2**. Seven subjects did not have remission after completing the induction phase (**Table 3**).

Five of the 7 subjects who did not achieve remission were from the abnormal BG group. However, there was no significant difference between remission status in the euglycemia and abnormal BG group (P=0.533) (**Table 4**). Bivariate analysis of potential predisposing factors (sex, age, risk stratification, and nutritional status) to BG during induction phase revealed that only nutritional status was significantly correlated with abnormal BG **(Table 5)**.

Multivariate logistic regression analysis was performed to to further study the correlation between abnormal BG and age and nutritional status, the two variables with P<0.25 in bivariate analysis. Age ≥ 10 years had no significant correlation with abnormal BG (P=0.396), however, undernourished nutritional status had a significant correlation with abnormal BG (P=0.005) (**Table 6**).

Discussion

The ALL survival rate has been improved to 90%.[12] Chemotherapy for ALL including induction, consolidation, and maintenance phases lasts for 2-3 years.[13] Two agents used in the induction phase, steroids and L-Asp, have been documented to cause hyperglycemia. The predominant mechanism of steroid-increased BG level is by induction of insulin resistance.[3,4] The risk of hyperglycemia increases when L-Asp is co-administered.[14] A study reported that the incidence of hyperglycemia as a side effect of steroid and L-Asp combination was 5.2%, from three centers in Indonesia.[15] We found that 6 subjects (16.6%) experienced hyperglycemia when they received prednisone as well as L-Asp simultaneously during the induction phase, with fasting BG ranging from 130 mg/dL to 311 mg/dL , and 329 mg/dL in one episode of two-hour postprandial BG. Native L-Asp, on the contrary, was reported to result in more hypoglycemia than hyperglycemia during

Table 2. Onset of abnormal BG during induction phase chemotherapy

Abnormal BG	Subjects	Weeks								BG level (range)
		0	1	2	3	4	5	6	7	
Hyperglycemia		2	3	1	-	-	-	-	-	Fasting: 130-311 mg/dL 2PP: 329 mg/dL
Hypoglycemia		2	-	7	-	-	-	-	-	Fasting: 40-64 mg/dL 2PP: 58 mg/dL
Hypoglycemia and hyperglycemia (subjects experiencing hypoglycemia then followed by hyperglycemia)	Subject I	hypo					hyper			Hypoglycemia: Fasting: 56-64 mg/dL 2PP: N/A Hyperglycemia: Fasting: 267 mg/dL 2PP: 211-388 mg/dL
	Subject II			hypo	hyper					
	Subject III		hypo hyper							
Hyperglycemia and hypoglycemia (subjects experiencing hyperglycemia then followed by hypoglycemia)	Subject I		hyper		hypo					Hyperglycemia: Fasting: 131-205 mg/dL 2PP: 211 mg/dL Hypoglycemia: Fasting: 53-63 mg/dL 2PP: N/A
	Subject II			hyper		hypo				
	Subject III				hyper	hypo				
	Subject IV	hyper			hypo					

Hyper=hyperglycemia; hypo=hypoglycemia; 2PP: 2 hours post prandial

Table 3. Blood glucose level during inductive phase chemotherapy and BMP after chemotherapy

Variables	(N=36)
Blood glucose levels, n	
Hypoglycemia	9
Hypoglycemia-hyperglycemia	7
Hyperglycemia	6
Euglycemia	14
Bone marrow puncture, n	
Complete remission	29
No remission	7

Table 4. The effect of abnormal BG on remission status

	No remission	Complete remission	OR (95%CI)	P value
Abnormal	5	17	1.765 (0.292 to 10.661)	0.533
Euglycemia	2	12		

Chi-square test

Table 5. Bivariate analysis of BG and potential predisposing factors

Variables	Euglycemia (n=14)	Abnormal (n=22)	OR (95%CI)	P value
Gender, n			1.600 (0.414 to 6.177)	0.494
Male	8	10		
Female	6	12		
Age, n			0.294 (0.069 to 1.249)	0.091
<10 years	7	17		
≥10 years	7	5		
Risk stratification, n			1.167 (0.269 to 5.054)	0.837
High	10	15		
Standard	4	7		
Nutritional status, n			27.857 (3.016 to 257.267)	0.000
Well-nourished	13	7		
Undernourished	1	15		

Table 6. Multivariate logistic regression analysis

	OR (95%CI)	P value
Age (>10 years)	0.468 (0.081 to 2.707)	0.396
Nutritional status (undernourished)	24.353 (2.590 to 228.958)	0.005

chemotherapy in childhood ALL.[7] Seven of 9 subjects in our hypoglycemia group suffered from hypoglycemia after the second dose administration of L-Asp in the second week, with fasting BG ranging from 40 mg/dL to 64 mg/dL, and 58 mg/dL in one episode of two-hour postprandial BG. L-Asp-induced hypoglycemia occurs through hyperinsulinism.[16,17] However, we did not examine subjects' insulin level. Seven subjects had hypoglycemia as well as hyperglycemia, with 3 subjects experiencing hypoglycemia first, followed by hyperglycemia, with fasting BG ranging from 56 mg/dL to 64 mg/dL; none of them had two-hour postprandial hypoglycemia, whereas BG in the hyperglycemia episode ranged from 211 mg/dL to 388 mg/dL two-hour postprandial, and 267 mg/dL in one episode of fasting hyperglycemia. The other 4 subjects had hyperglycemia then hypoglycemia, with fasting BG ranging from 131 mg/dL to 205 mg/dL, and 211 mg/dL in one episode of two-hour postprandial BG. While BG in the episodes of fasting hypoglycemia ranged from 53 mg/dL to 63 mg/dL, and no episode of hypoglycemia in two-hour postprandial BG. Fourteen others had normal BG throughout induction phase.

Bivariate analysis revealed that age 10 years and over as well as undernourished nutritional status were significantly correlated with abnormal BG. This finding was in line with a study which noted that the most important predictor of hyperglycemia in pediatric ALL during chemotherapy was age older than 10 years.[18] However, upon further multivariate regression analysis in our study, age >10 years had no significant correlation with abnormal BG. Nevertheless, undernourished nutritional status was significantly correlated with abnormal BG in our multivariate analysis. A previous study discovered that malnourished children experienced insulin resistance affecting BG.[19]

A study reported that ALL subjects who experienced hyperglycemia during induction phase chemotherapy had lower 5-year overall and relapse-free survival than those with euglycemia.[6] However, to our knowledge there have been no studies about the effect of hypoglycemia on survival rate. In our study, all subjects were followed up until the BMP evaluation was performed in the end of induction phase, revealing that 7 subjects (19.4%) did not achieve remission, 5 of whom were from abnormal BG group.

The study limitations were not measuring subjects' total caloric intake per day and lack of data about family history of diabetes mellitus. Since our study only covered the induction phase of 8 weeks, we cannot describe the final outcomes of our subjects. In conclusion, abnormal BG in children with ALL undergoing induction phase chemotherapy has no significant association with remission at the end of induction phase, and undernourishment is correlated with abnormal BG in childhood ALL patients.

Conflict of interest

None declared.

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Original Article

Implementation of *Dengue Recurrent Shock Prediction Score* in pediatric dengue shock syndrome

Armand Setiady Liwan, I Wayan Gustawan, Eka Gunawijaya, Soetjiningsih, Ketut Ariawati, I Nyoman Budi Hartawan

Abstract

Background Global morbidities due to dengue viral infection increase yearly. The pediatric mortality rate from dengue shock syndrome (DSS) remains high. Early identification of the risk of recurrent shock may serve to increase awareness and reduce mortality. *The Dengue Recurrent Shock Prediction Score* (DRSPS) is a tool to predict recurrent shock in children with DSS, but the optimal cut-off point in our population is still unknown.

Objective To assess the validity of the DRSPS by determining the optimal cut-off point that can be used in Indonesia

Methods This cross-sectional prospective study was done at Sanglah Hospital, Denpasar, Bali. Risk of reccurent shock were classify based on DRSPS in all DSS patient, and they were observed whether they will experienced recurrent shock or not.

Results Of 56 children with DSS, 27 subjects had recurrent shock and 29 subjects did not. The optimal DRSPS cut-off point was -189.9 for predicting recurrent shock, with 87.4% area under the curve (AUC), 81.5% sensitivity and 82.8% specificity.

Conclusion The optimal cut-off point of DRSPS was -189.9 and it has good validity. The results of this study are expected not only to be used as the basis for further study, but to increase physician awareness in treating DSS patients. **[Paediatr Indones. 2020;60:178-85 ; DOI: 10.14238/pi60.4.2020.178-85]**.

Keywords: *dengue shock syndrome; recurrent shock; DRSPS*

The national mortality rate of dengue hemorrhagic fever (DHF) declined from 41.4% in 1968, to 4% in 1980, to 1.4% in 2000, and to only 0.88% in 2012. However, the number of deaths due to dengue shock syndrome (DSS) remain high. Data from six teaching hospitals in Indonesia in 2008-2013 revealed that the mortality rate due to DSS was about 7.81%, reaching 10-20% if proper initial treatment is lacking.1 Hence, we need to better identify the risk factors of shock in children with dengue infection, with the aim of increasing physician awareness for stricter supervision of patients.[1,2]

Deaths from dengue infections were reported to be fifty times higher in patients with shock than those without shock. About 30% of shock patients experience recurrent shock, affecting subsequent treatment and outcomes. In 2016, there were 245 pediatric DSS cases in RSUP Sanglah, Bali, of whom 119 cases (48.5%) had recurrent shock.[2] Recurrent shock generally occurs during the critical phase of

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dengue infection. Some patients experience several episodes of shock within 24-48 hours before the convalescent period. Risk factors for recurrent shock of DSS include young age, earlier onset of shock, higher body temperature, faster pulse, higher hematocrit rate, and poor hemodynamic status.[2,3-4]

Early identification of the risk factors for recurrent shock can help increase awareness to prevent recurrence of shock episodes in children with DSS. Huy *et al.*[3] in Vietnam developed a model to predict the occurrence of recurrent shock in children with DSS. This model includes physical and laboratory examination results, with 68.3% sensitivity and 68% specificity. *The Dengue Recurrent Shock Prediction Score* (DRSPS) can yield differing results when applied to the population in Indonesia, not only because of the climate difference, but also because of variations in local expertise and health facilities in Indonesia and Vietnam.[3-5]

Despite these differences, the practical reasons for assessing the validity of the Huy's model in Indonesia are: the high mortality rate of DSS in children, the difficulty of identifying the risk of recurrent shock in children with DSS as there is no standard method to predict recurrent shock in children with DSS in Indonesia, and the lack of validity studies on Huy's model (or DRSPS). As such, we aimed to assess the validity of the DRSPS by determining the optimal cut-off point that can be used in Indonesia.

Methods

This diagnostic study was done to determine the optimal DRSPS cut-off point to predict recurrent shock in children with DSS. Patients at Sanglah Hospital, Bali, were selected by consecutive sampling from January 2019 until the minimum required sample size was fulfilled. The inclusion criteria were children aged ≥6 months to 18 years with DSS diagnosed by the 2011 WHO criteria,[6] and whose previous shock episode had been resolved with a maximum of 2 crystalloid bolus administrations at 20 mL/kg body weight. Exclusion criteria were patients with chronic clinical conditions or massive bleeding that required blood transfusions. The minimum required sample size of 56 subjects was based on a 48.5% prevalence of recurrent shock in DSS.

At the time of initial diagnosis, all subject were classified into compensated and decompensated shock. Subjects' data were documented on the DRSPS form. The DRSPS included several components: duration of fever before admission, purpura/ecchymosis, ascites/pleural effusion, platelet count at shock, and pulse pressure during shock. Each component had its own corresponding value **(Table 1)**. The total corresponding value of each sample was analyzed to find the optimal cut-off point. Based on new optimal cut-off point, subjects were grouped into either the risk of recurrent shock group or the no risk of recurrent shock group. Subjects were followed until they were discharged or died, whether they had recurrent shock or not. The outcomes of this study were a new optimal cut-off point based on sample population, AUC value, and accuracy (sensitivity, specificity; positive and negative predictive value) based on the original and new optimal cut-off point.

Table 1. Dengue recurrent shock prediction score (Huy's model)[3]

Variables	Results	Score
Fever duration before admission (in days)		
Purpura/ecchymosis		
Ascites/pleural effusion		
Platelet count at shock (/μL)		
Pulse pressure at shock		
Total		

Notes: Fever duration before admission (-40 per day), purpura/ecchymosis (+50 if positive), ascites/pleural effusion (+150 if positive), platelet count at shock (-7 per 10,000 platelets), pulse pressure at shock (-4 per 1 mmHg). Total score >-154.5 (Huy's cut-off) was considered to be at risk of recurrent shock.

The characteristics of subjects were presented in tables and narration. The optimal cut-off point analysis was presented in graphs and tables. The accuracy of DRSPS was assessed by 2x2 cross-tabulation. The data obtained were analyzed by *SPSS Statistic 22*. This study was approved by the Research Ethics Committee of the Universitas Udayana Medical School/Sanglah Hospital, Denpasar.

Results

During the study period, 56 DSS patients met the inclusion criteria. Subjects' mean age was 9 years,

ranging from 2-16 years. Subjects' mean length of hospitalization was 3 days; two subjects required prolonged hospitalization of up to 14 and 15 days due to secondary bacterial infections. At the initial diagnosis, most subjects were in the compensated DSS group (51%). Recurrent shock prevalence was 48.2%, and 15 out of 27 recurrent shock subjects were in the decompensated DSS group. Most children experienced the first shock after a 4-day fever, ranging from 2 to 6 days of fever. Subjects' mean platelet count was 35x103/μL, ranging from 21 to 44x103/μL. The most common difference in pulse pressure in our subjects was 20 mmHg, with a mean of 14 mmHg. The basic characteristics of subjects are shown in **Table 2**.

Table 2. Subjects' characteristics

Characteristics	Recurrent shock	
	Yes (n=27)	No (n=29)
Gender, n		
Male	17	10
Female	17	12
Age, n		
Toddler	3	2
Preschool	4	3
School-age	9	9
Teenager	11	15
Nutritional status, n		
Mild-moderate malnutrition	5	3
Well-nourished	17	14
Overweight	1	3
Obese	4	9
Ascites/pleural effusion, n		
Yes	12	29
No	15	0
Purpura/ ecchymosis, n		
Yes	13	20
No	14	9
Length of stay, n (%)		
<3 days	5	4
3-5 days	19	22
>5 days	3	3
Outcomes, n		
Died	5	0
Survived	22	29

Using the numeric DRSPS of subjects in **Table 3**, we calculated the optimal cut-off point for the local population to be -189.9. It was started from the optimal cut-off point formed in **Figure 1**, then the pseudo line was drawn perpendicular down and showed exactly number 29. The ROC curve had 87.4% AUC, with 81.5% sensitivity and 82.8% specificity (**Figure 1** and **Figure 2**). The diagnostic value of DRSPS using the original and new optimal cut-off point in predicting the occurrence of recurrent shock is shown in **Table 4**. Based on cross-tabulation data in **Table 4**, we calculated several accuracy parameters of DRSPS. The accuracy of this model using both cut-off point is shown in **Table 5**.

The value of diagnostics depended not only on sensitivity and specificity, but also the predictive value based on the prevalence of the diseases. Low prevalence in a population could lead to a high level of false positive, so more specific tests were needed. The higher the prevalence of a disease, the higher the positive predictive value (PPV). When the prevalence of the diseases dropped below 45%, it showed that the PPV based on optimal cut-off also dropped below 80%. **Figure 3** shows the effect of the prevalence on positive predictive value when DRSPS was applied to different populations's prevalence.

Discussion

In the last three decades, there has been an increase in the frequency of dengue infections worldwide. The number of cases of dengue infection has continued to increase since the 1950s. In 2008 there has been an increase of almost ¾ times from the previous decade. Around 2.5 billion of the world's population lives in dengue endemic areas and more than half are in 10 countries in Southeast Asia. There were 156,052 cases of dengue infection with 1396 deaths occurred in Indonesia and the highest number of deaths in Southeast Asia. In 2016, there were 245 pediatric DSS cases in RSUP Sanglah, Bali, of whom 119 cases (48.5%) had recurrent shock. Some patients experience several episodes of shock within 24-48 hours before the convalescent period. Early identification of the risk factors for recurrent shock can help increase awareness to prevent recurrence of shock episodes in children with DSS. A simple model which found by Huy *et al.*[3] can be used to predict the occurrence of recurrent shock in children with DSS. In this study we try to assess the validity of the DRSPS by determining the optimal cut-off point that can be used in Indonesia.[1,2,6,8]

Table 3. Sensitivity and specificity of each subject

No	Value	Sensitivity	Specificity	No	Value	Sensitivity	Specificity
1	-315.3	1	0	29	-189.9	0.815	0.828
2	-308.65	1	0.034	30	-188.75	0.815	0.862
3	-298.765	1	0.069	31	-179.35	0.815	0.897
4	-294.16	0.963	0.069	32	-170.35	0.778	0.897
5	-293.545	0.963	0.103	33	-152.9	0.741	0.897
6	-292.05	0.963	0.138	34	-133.95	0.704	0.897
7	-289.9	0.963	0172	35	-130.9	0.667	0.897
8	-287.8	0.963	0.207	36	-128.05	0.63	0.897
9	-283.25	0.963	0.241	37	-125	0.593	0.897
10	-272.8	0.963	0.276	38	-122.9	0.593	0.931
11	-263.7	0.963	0.31	39	-121.865	0.556	0.931
12	-261.35	0.926	0.31	40	-119.565	0.556	0.966
13	-259.075	0.926	0.345	41	-115.45	0.519	0.966
14	-255.875	0.926	0.379	42	-110.2	0.481	0.966
15	-254.25	0.926	0.414	43	-105.05	0.444	0.966
16	-252.95	0.926	0.448	44	-102.2	0.407	0.966
17	-251.915	0.926	0.483	45	-99.265	0.37	0.966
18	-248.055	0.926	0.517	46	-77.815	0.37	1
19	-237.54	0.926	0.552	47	-54	0.333	1
20	-228.25	0.889	0.552	48	-46.9	0.296	1
21	-223.88	0.889	0.586	49	-39.85	0.259	1
22	-220.895	0.889	0.621	50	-28.1	0.222	1
23	-218.23	0.889	0.655	51	-19.2	0.185	1
24	-213.215	0.889	0.69	52	-5.4	0.148	1
25	-206.4	0.889	0.724	53	22.3	0.111	1
26	-201.82	0.889	0.759	54	37.525	0.074	1
27	-197.07	0.852	0.759	55	73.175	0.037	1
28	-191.8	0.815	0.793	56	109.8	0	1

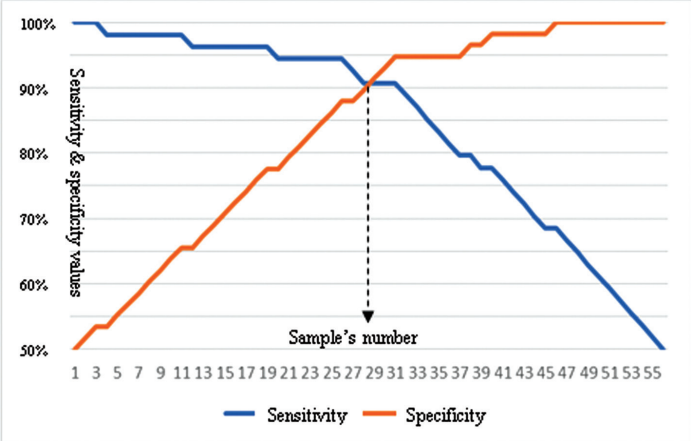

Figure 1. Optimal cut-off point

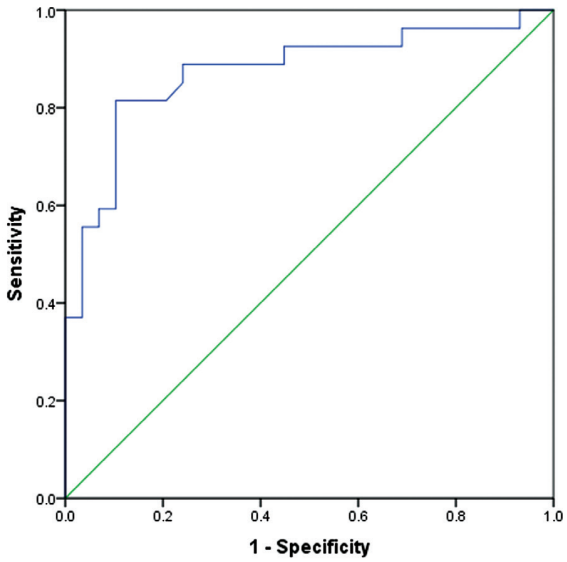

Figure 2. ROC curve

Table 4. Cross-tabulation of DRSPS

DRSPS	Recurrent shock (Optimal cut-off -189.9)			Recurrent shock (Original cut-off)		
	Yes	No	Total	Yes	No	Total
Risk	22	5	27	23	3	26
No risk	5	24	29	4	26	30
Total	27	29	56	27	29	56

Table 5. Diagnostic values of dengue recurrent shock prediction score

Values	Accuracy	
	Optimal cut-off -189.9 (%)	Original cut-off (%)
Sensitivity	81.5	85.2
Specificity	82.8	89.7
Positive predictive value	81.5	88.5
Negative predictive value	82.8	86.7

A previous study reported that the prevalence of recurrent shock was 28% in children with DSS in Vietnam.[3] However, another study reported 48.5% prevalence in Bali,[2] similar to a previous study (48.3%).[7] In addition, a Vietnam study reported 35.9% prevalence of recurrent shock.[5] The Vietnamese studies had slightly different results, perhaps because Lam *et al.*[5] sourced their sample population from one hospital, while Huy *et al.*[3] sourced their sample from public health centers in a small province and referral center hospitals in major cities. In our study, 27 of 56 children suffered recurrent shock (48.2%). Two Indonesian studies had similar results, probably because they were conducted in referral center hospitals and were located in regions of Indonesia, which has a tropical climate.[4,6] In fact, Tarigan *et al.*[2] study was done in our institution, Sanglah Hospital (referral center hospital). In addition, the Indonesian studies reported higher prevalences compared to the Vietnamese studies. This difference may have been

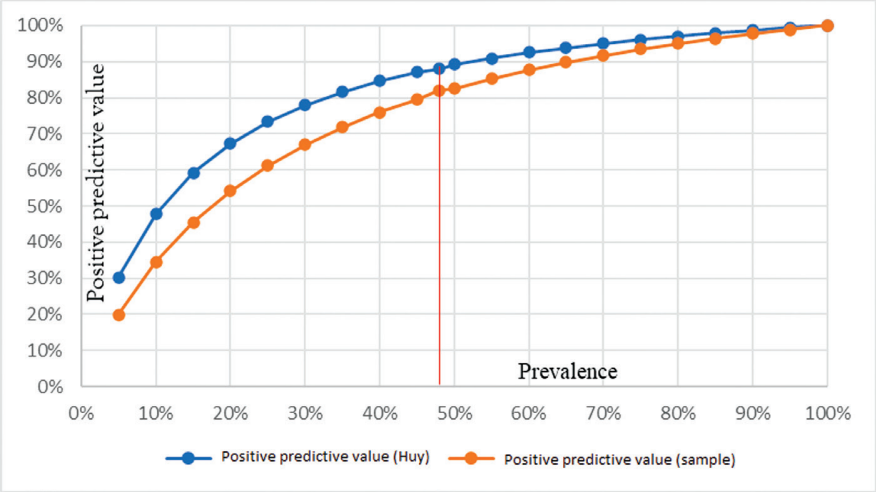

Figure 3. Prevalence effect on positive predictive value

due to (subtropical), regional climate differences (tropical *vs.* sub-tropical, respectively). Warmer air temperatures in tropical regions have been associated with increased spread of dengue viruses, as they directly affect the life cycle of *Aedes aegypti* and *Aedes albopictus* mosquitoes which are the primary vectors of dengue virus transmission.[6-8]

Most children in our study experienced the first shock after a 4-day fever, with the earliest shock occurring after 2 days of fever. Fever is one of five variables of the DRSPS, with one day of fever contributing a negative value of 40 points. Huy *et al.*[3] noted that shorter fever duration before 1st shock relates to the risk of recurrence of shock in children with DSS. Since DSS generally occurs after 4 days of fever, an earlier onset of shock indicates a higher likelihood of severe disease and risk of recurrent shock events.[5,7]

A previous study developed a model for predicting mortality in severe dengue cases. The states of lethargy, bleeding, increased heart rate, decreased bicarbonate levels, and elevated lactate levels were predictors of death in severe dengue cases, but the best predictive model of mortality was obtained by combining the bicarbonate rate with ALT (AUC 83.5%).[9] Another study found that albumin could be used as a predictor of shock in dengue patients (OR 17.4; AUC 86.5%; sensitivity 79%; specificity 81%), but not as a predictor of recurrent shock in DSS.[6] Our independent variables (DRSPS) differed from variables in the two aforementioned studies.[6,9] In addition, the sample was comprised of adult patients in a previous study[9] making it difficult to compare to the variables in the Lam and Huy's models, as children have different characteristics and immunity from adults.

The DRSPS, a simple model developed by Huy *et al.*[3] has relatively good precision with an AUC of 73%, sensitivity of 68.3%, and specificity of 68.2%. Another model by Lam *et al.*[5] in Vietnam to predict recurrent and prolonged shock had an AUC of 69%. They applied the DRSPS to their study population and obtained a relatively low AUC of 54%.[5] The Huy *et al.*[3] and Lam *et al.*[5] studies reported purpura/ecchymosis in 36% and 3% of subjects, respectively. The proportions of the ascites/pleural effusion also differed (44% *vs.* 1%, respectively).[3,5]

Our validation of the DRSPS showed different results. The new optimal cut-off point of -189.9 from our sample population had 81.5% sensitivity, 82.8% specificity, and 87.4% AUC. It was difficult to compare the validity of DRSPS of this study with the study by Huy due to the distinct prevalence of recurrent shock. In addition, the proportions of pleural effusion and purpura/ecchymosis differed from the Huy *et al.*[3] study. The proportions of the ascites/pleural effusion in our study was 58.9%, and the proportions of ascites/pleural effusion 73,2%. These two variables in DRSPS score (Huy's model) which gives a positive significance of recurrent shock (+50 if purpura/ ecchymosis was occured, and +150 if ascites/pleural effusion was occured).

The original cut-off and our optimal cut-off differed by 35.4 points, leading to a different positive predictive value of 81.5%. The value of type I error or false positive (α) with original cut-off was 5.4%, while that of our optimal cut-off point was 8.9%. This finding indicates that up to 2 children in 100 would be falsely positive for risk of recurrent shock. The type II error or false negative (β) with original cut-off was 7.1%, while that of our cut-off point was 8.9%. This finding indicates that 1 child out of 100 not predicted to experience recurrent shock, will experience it.[11]

The accuracy of the DRSPS depends not only on the sensitivity and specificity, but also on the prevalence of diseases in a population. The higher the prevalence of a disease, the higher the positive predictive value. This model applied to a place with differing prevalence will yield a different result. In populations with a low prevalence of recurrent shock, the DRSPS could lead to high false positive values and low positive predictive values, whereas in populations with a high prevalence of recurrent shock, more sensitive tests are needed than specific tests. With a decrease in prevalence of <35%, the positive predictive value (based on our optimal cut-off point) begin to decline below 70%. The prevalence of recurrent shock was 48.2%, indicating that positive predictive values remained above 80% for both cut-offs. We expect that response times would differ, as Huy *et al.*[3] did not report the time of first fluid administration. The level of parental understanding, awareness, and education in different populations may also reveal different values. The most common dengue serotype in the two Vietnamese studies was DENV-2.[3,5] We did not identify serotypes, which may have differed from the Vietnamese studies. Balinese dengue serotypes are reportedly predominantly DENV-3, followed by DENV-1, DENV-2, and DENV-4.[12] Other limitations were that subjects were diagnosed solely based on 2011 WHO criteria without further investigation of dengue serotypes/PCR examination; timing of the initial fluid resuscitation was not documented; and subjects who died before the seventh day of fever onset did not undergo anti-dengue serological examinations.

In conclusion, the DRSPS can be used to predict recurrent shock in children with DSS. Without underestimating the need for clinical judgment, the DRSPS can be used in daily practice to increase doctor awareness. Therefore, it is expected to reduce morbidity and mortality from recurrent shock in children with DSS.

Conflict of Interest

None declared.

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Original Article

Achievement of full enteral feeding using volume advancement in infants with birth weight 1,000 to <2,000 grams

Teti Hendrayani, Afifa Ramadanti, Indrayady, Raden Muhammad Indra

Abstract

Background Early enteral feeding is one of the efforts to improve gastrointestinal adaptability in preterm infants. Volume advancement (VA) enteral feeding has been associated with less time to reach full feeding, which can improve outcomes.

Objective To evaluate the duration of VA needed to achieve full enteral feeding (FEF) in low birth weight (LBW) and very low birth weight (VLBW) infants and related factors.

Methods This prospective study was done in infants with birth weight 1,000 to <2,000 grams in the Neonatal Ward and NICU of Dr. Moh. Hoesin General Hospital, Palembang, South Sumatera. All infants underwent VA feeding. The time needed to achieve FEF (150 mL/kg/day) was recorded. Several clinical factors were analyzed for possible associations with the success rate of achieving FEF within 10 days of feeding.

Results Thirty-five infants were included in this study with a mean gestational age of 31.83 (SD 2.67) weeks. Their median body weight at the start of protocol was 1,400 (range 1,000-1,950) grams and 80% had hyaline membrane disease. Median time to achieve FEF was 11 (range 8-21) days, with 48.6% subjects achieving FEF in ≤10 days. Gestational age <32 weeks (OR 5.404, 95%CI 0.963 to 30.341), birth weight <1,500 grams (OR 5.248, 95%CI 0.983 to 28.003), and male gender (OR 4.751, 95%CI 0.854 to 26.437) were associated with the failure of achieving FEF within 10 days of feeding, however, no factors remained statistically significant after multivariate analysis.

Conclusion Full enteral feedings in infants with birth weight 1,000 to <2,000 grams with VA feeding are achieved within a median of 11 days. Gestational age, birth weight, and gender are not associated with time needed to achieve FEF. **[Paediatr Indones. 2020;60:173-7 ; DOI: 10.14238/pi60.4.2020.173-7]**.

Keywords: *LBW; VLBW; preterm infants; volume advancement; full enteral feeding*

Low birth weight (LBW) is defined as birth weight lower than 2,500 grams, and further classified into very LBW (VLBW), birth weight <1,500 grams and extremely (ELBW), birth weight <1,000 grams. Depending on gestational age, infants are grouped as moderate to late preterm, very preterm, and extremely preterm.[1,2]

More than 20 million infants worldwide representing 15.5% of all births are born with low birth weight, 96.5% of them in developing countries.[3] In 2013, the incidence was 10.2% in Indonesia and 9% in South Sumatera.[4] According to medical records at Dr. Moh. Hoesin General Hospital (RSMH) in 2017, 377 infants hospitalized in the NICU and Neonate Ward had body weight of 1,000 grams to < 2,000 grams (data not published).

Preterm infants have a high risk of mortality due to their immature organs, including gastrointestinal

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problems, as well as immature oromotor function, such as the lack of sucking and swallowing reflex coordination. These problems may cause difficulty in achieving FEF in LBW and VLBW infants, predisposing them to malnutrition. Two previous studies showed that VLBW infants are at risk of developmental delay, emphasizing the need for long-term neurodevelopmental follow-up.[1,5,6]

Enteral feeding for LBW infants typically starts at 10-15 mL/kg BW/day, with breast milk or formula for preterm infants. There are two enteral feeding strategies: frequency advancement (FA) and volume advancement (VA). In the VA method, feeding is characterized by rapidly increasing administered volume in increments of 20 mL/kg BW/day, while in the FA method, the frequency of administration is increased before volume. The goal of enteral feeding is to achieve full enteral feeding (FEF) of 150 mL/kg BW/day.[7-10] Early achievement of FEF (within 10 days) is associated with fewer nutritional complications.[11] Several factors may influence the achievement of FEF in LBW and VLBW infants, including gestational age, birth weight, APGAR score at 5 minutes, weight at feeding initiation, type of milk, and infection.[11]

This study was done in LBW and VLBW infants with weights of 1,000 grams to < 2,000 grams, as a pilot study, to identify the time required to achieve FEF (150 mL/kgBW/day) using VA enteral feeding. We also evaluated other factors potentially affecting FEF time.

Methods

This prospective study was conducted from October 2018 to February 2019 in Neonatal Ward and NICU of Mohammad Hoesin Hospital (RSMH), Palembang, South Sumatera. Hospitalized infants with birth weight 1,000 to < 2,000 grams were screened for inclusion after parents provided written, informed consent. Infants with major congenital malformations, malformation of the gastrointestinal tract, intraventricular hemorrhage grades 3 and 4, hypoxic ischemic encephalopathy, or incomplete infant data (no APGAR score, no birth data, such as birth weight) were excluded from this study.

All stable infants were enrolled and given 10 mL/kg/day in three hourly enteral feedings within 24-48 hours after birth. Volume was increased gradually with 20 mL/kg/day increments based on clinical condition. Full enteral feeding (FEF) was defined as the ability to tolerate 150 mL/kg/day of enteral feeding. Feeding consisted of expressed breast milk or premature formula by nasogastric tube. Before achieving FEF, additional parenteral nutrition was given to meet nutritional requirements. Infants experiencing clinical deterioration that necessitated cessation of enteral feeding for >24 hours were considered to have dropped out. And for unstable infants, who had respiratory distress, hypoglycemia, hypotension and shock, had late enteral feeding.

We also analyzed factors that may affect the time needed to achieve FEF, including gestational age, weight at the start of enteral feeding, presence of infection (clinical sepsis, sepsis, or bronchopneumonia), and respiratory distress syndrome.[9,11] Clinical sepsis was defined as general signs or symptoms of weakness, feeding problems, weight loss, lethargy, apnea attacks, dyspnea, cyanosis, diarrhea, vomiting, or circulatory or hematological disorders. Sepsis was defined as signs and symptoms of clinical sepsis with two or more laboratory findings: leukocyte < 5,000/mm^3 or > 34,000/mm^3, ratio of immature to total neutrophils of 0.2 or more, ESR > 15 mm/hours, and C-reactive protein > 9 mg/dL. Bronchopneumonia was defined as breathing difficulties characterized by dyspnea, tachypnea, chest retractions, respiratory grunting, cyanosis with normal or decreased respiratory sounds, crackles, rales, and infiltrates on chest x-ray.[2]

This study was reviewed and approved by the Ethics Committee of Sriwijaya University Medical Faculty. Bivariate analysis was done using Chi-square test and multivariate analysis using logistic regression test. Statistical analyses were carried out using *SPSS for Windows version 24.0*.

Results

Forty-two infants were screened for our study, but seven dropped out from study because of clinical deterioration [NEC (2 infants), respiratory failure due to pulmonary hemorrhage and subsequent death (2) duodenal stenosis (1), and gastrointestinal bleeding leading to cessation of enteral nutrition more than 24 hours (2)]. Hence, a total of 35 infants were analyzed (**Table 1**).

Table 1. Clinical characteristics of subjects

Characteristics	(N=35)
Gestational age, n	
Extremely preterm (< 28 weeks)	1
Very preterm (28 to < 32 weeks)	14
Moderate to late preterm (32 to < 37 weeks)	20
Birth weight, n	
1000 - < 1500 grams	21
1500 - < 2000 grams	14
Gender, n	
Male	19
Female	16
APGAR score at 5 min, n	
< 5	1
5 or more	34
Body weight at start of protocol, n	
1,000 to < 1,500 grams	21
1,500 to < 2,000 grams	14
Type of milk, n	
Breast milk	14
Formula milk	21
Infection, n	
Yes	7
No	28
Birth place, n	
RSMH	27
Others	8
Time to start enteral feeding, n	
< 24 hours	9
24-48 hours	26

Birth weight was identical to weight at initiation of enteral feeding in all infants, with median 1,400 (1,000-1,950) grams. Mean gestational age was 31.83 (SD 2.67) weeks. Hyaline membrane disease was found in 80% of infants. Mean FEF achievement was 11.7 days, with median 11 (range 8-21) days. There were 17 (48.6%) subjects who achieved FEF in ≤ 10 days. Mean weight gain was 5.8 grams each day, with median weight gain 5.69 (range -2.20-18.67) grams/kg/day.

Factors potentially affecting achievement of FEF are listed in **Table 2**. Subjects with gestational age less than 32 weeks, body weight less than 1,500 grams, and male gender had greater proportions of failure to achieve FEF within 10 days. Correlation analysis identified a moderately negative correlation between weight gain and starting weight (**Table 3**). After multivariate analysis, no factor was found to independently influence FEF achievement (**Table 4**).

Discussion

In this prospective study on infants with birth weight of 1,000 to <2,000 grams, we aimed to assess the rate of full enteral feeding achievement. We found that only 48.6% of infants successfully achieved FEF in < 10 days. The median time needed to achieve FEF in all subjects was 11 days. No complications related to enteral feeding were apparent in this study. Not achieving FEF in < the first 10 days of life has been associated with malnutrition (odds ratio 1.97).12 Another study found that with either volume advancement or frequency advancement, infants regained their birth weight within 7-10 days. The longer duration needed for FEF achievement in our study may have been related to younger gestational age, which is associated with gastrointestinal tract immaturity and inadequate digestive enzymes.9

We noted a mean weight increase of 5.8 grams/kg/day. This result was lower than a previous study on low birth weight infants. They reported that 60% could achieve weight gain of >5 grams/kg/day, and risk factors for not gaining more weight included gestational age, birth weight, intrauterine growth, and complicating illnesses.13 In our study, the overall lower weight gain may have been related to the finding that the majority of subjects had complicating illnesses.

Factors affecting the success in achieving FEF in this study were gestational age, and starting weight. Younger gestational age (< 32 weeks), lower starting weight (1,000 to < 1,500 grams), and male sex were associated with failure to achieve FEF within 10 days of enteral feeding. A study found that infants with weight < 1,000 grams needed 2 weeks to achieve FEF, while those with weight between 1,000 and 1,500 grams only needed one week.7 Another study estimated that an additional one week of gestational age would reduce the time needed to achieve FEF by 15.9%. The same study also found that small for gestational age can increase the time needed to achieve FEF by 16.6%.11 Previous studies found that male sex was associated with worse short and long term outcomes, usually associated with infections.7,9 No factors in this study remained significant upon multivariate analysis, indicating that interplay between factors may be needed to affect the outcome.

Factors associated with success in achieving FEF in another study, but not ours, were higher CRIB II

Table 2. Bivariate analysis of factors potentially affecting achievement of FEF within 10 days enteral feeding (N=35)

Variables	Not achieved	Achieved	Total	RR	95%CI	P value
Gestational age						
< 32 weeks	11	4	15	2.095	0.167 to 1.008	0.025
32 or more weeks	7	13	20			
Body weight at start of protocol						
1,000 to < 1,500 grams	14	7	21	2.33	0.234 to 0.930	0.027
1,500 to < 2,000 grams	4	10	14			
Gender						
Male	12	7	19	1.684	0.293 to 1.188	0.130
Female	6	10	16			
Type of milk						
Formula milk	10	11	21	0.952	0.478 to 1.898	0.890
Breast milk	7	7	14			
Infection						
Yes	4	3	7	1.143	0.545 to 2.397	0.735
No	14	14	28			
Clinical sepsis						
Yes	2	1	3	0.627	0.123 to 3.212	0.478*
No	15	17	32			
Sepsis						
Yes	0	3	3	2.133	1.475 to 3.085	0.125*
No	15	17	32			
Bronchopneumonia						
Yes	2	1	3	0.427	0.047 to 4.744	0.478*
No	17	15	32			
HMD						
Yes	17	14	31	2.194	0.390 to 12.338	0.261*
No	1	3	4			

*Fisher's exact test

Table 3. Correlation with weight gain per day

Variables	r	P value
Body weight at start of protocol	-0.531	0.001
Gestational age	-0.317	0.063

Table 4. Regression logistic analysis for factors potentially affecting achievement of FEF within initial 10 days of enteral feeding

Variables	OR	95%CI	P value
Gestational age < 32 weeks	5.404	0.963 to 30.341	0.055
BW < 1,500 grams	5.248	0.983 to 28.003	0.052
Gender	4.751	0.854 to 26.437	0.075

scores, maternal hypertension, Caesarean delivery, formula milk, presence of patent ductus arteriosus, and complicating illnesses such as respiratory distress syndrome, sepsis, and pneumonia.11 The lack of association in our study may have been due to the small sample size. Moreover, the majority of subjects in our study had respiratory distress syndrome, which may mask its effects and the effects of other illnesses. In conclusion, infants with weight 1,000 to < 2,000 grams who receive volume advancement enteral feeding, have a median duration of 11 days to achieve FEF, which is slightly longer than the recommended duration of 10 days. We also noted VA enteral feeding to be safe. Infants with younger gestational age, lower starting weight, and male sex tend to achieve FEF in more than 10 days. We recommend a larger study to better characterize factors affecting the success of achieving FEF using VA enteral feeding.

Conflict of interest

None declared.

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Original Article

Do low vitamin D levels facilitate renal parenchymal injury?

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Abstract

Background Decreased vitamin D levels lead to an increase in infectious diseases, including urinary tract infections (UTIs).

Objective To assess serum vitamin D levels in children with renal parenchymal injury secondary to UTIs.

Methods Forty-three upper UTI patients and 24 controls, aged 1-15 years, were included. Vitamin D levels and other laboratory tests were obtained when they first admitted to hospital. 99mTc-labeled dimercaptosuccinic acid (DMSA) scans were performed to evaluate renal parenchymal injury.

Results Mean serum 25-hydroxyvitamin D (25(OH)D) was lower in the upper UTI group compared to the control group [18 (SD 9) *vs*. 23 (SD 10.6) ng/mL, respectively; P=0.045]. The upper UTI group was sub-divided into two groups, those with 22 (51.1%) and without 21 (48.8%) renal parenchymal injury. Mean 25(OH)D was significantly lower in patients with renal parenchymal injury [15.1 (SD 7.1) *vs*. 21 (SD 9.9) ng/mL, respectively; P=0.03]. The renal parenchymal injury cases were further sub-divided into two groups: 8 patients (36.3%) with acute renal parenchymal injury and 14 (63.6%) with renal scarring (RS), but there was no significant difference in 25(OH)D between these two groups [12.5 (SD 8.9) *vs*. 16.6 (SD 5.7) ng/mL, respectively; P=0.14).

Conclusion Decreased vitamin D is associated with renal parenchymal injury in children with upper UTIs. However, vitamin D is not significantly decreased in renal scarring patients compared to acute renal parenchymal injury patients. **[Paediatr Indones. 2020;60:205-11 ; DOI: 10.14238/pi60.4.2020.205-11]**.

Keywords: *child; upper UTI; renal injury; vitamin D*

The prevalence of urinary tract infections (UTIs) is 7.8% in children and adolescents.[1] After infancy, it decreases in boys, while it increases in girls.[2] The complications of UTI have long been known, one of which is renal scarring (RS), which develops in 15-60% of children with upper UTIs.[3] As the number of pyelonephritis events increases, the risk of RS increases concomitantly.[4] The 99mTc-labeled dimercaptosuccinic acid (DMSA) scan is the gold standard method for diagnosing RS.[5] Extensive RS may progress to hypertension, proteinuria, and end-stage renal disease.[6]

Vitamin D is a vital hormone, primarily necessary for calcium homeostasis and bone health. Vitamin D deficiency is defined as less than 20 ng/mL of serum 25-hydroxyvitamin D25[(OH)D].[7] Vitamin D also stimulates cathelicidin synthesis. These molecules have direct antimicrobial effects against many different microorganisms.[8] Observational studies have shown that vitamin D deficiency plays an important

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role in respiratory tract infections, sepsis, UTIs, and hepatic fibrosis.[9-12] The aim of this study was to investigate for a potential relationship between vitamin D and renal parenchymal injury in children with UTIs.

Methods

This prospective study included pediatric patients admitted to our hospital between April 2016 and November 2017 with a diagnosis of upper UTI. Diagnoses were established in patients with complaints like fever, abdominal pain, anorexia, and nausea, as well as positive urine cultures.[5,13,14] Urine samples were collected with a plastic bag or urinary catheter in patients under 2 years of age; midstream urine samples were obtained from older children. Only urine culture-positive patients (≥105 bacterial colony growth in 1 mL of culture in a midstream clean catch or sterile plastic bag, or ≥104 colony count by catheter) were included. Individuals with signs of puberty were accepted as adolescents. Children with ≥2 upper UTIs during follow-up were considered to have recurrent UTI.

The DMSA scanning was ordered to evaluate renal parenchymal injury, and performed no later than 2 weeks after hospital admission.[15] The DMSA was requested only once for each patient. Acute renal parenchymal injury was defined as the presence of focal, multifocal, or diffuse decreased DMSA uptake in the involved kidney without volume loss.[16] Renal scarring was defined as the presence of focal or multifocal decreased DMSA uptake and loss of volume in the involved renal cortex.[16] The DMSA images were investigated by a nuclear medicine specialist. Voiding cystourethrogram (VCUG) was ordered, with the latter performed after completion of antibiotic treatment.

At time of admission white blood cells (WBC), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were performed. Neutrophil-to-lymphocyte ratio (NLR) was calculated as the ratio of absolute neutrophil count (ANC) to absolute lymphocyte count (ALC). Serum 25(OH)D level was a good indicator of vitamin D status because its half-life is as long as 2-3 weeks.[17] Serum specimens of 25(OH)D collected from patients were frozen at -80°C. Serum 25(OH)D level was measured by enzyme-linked immunosorbent assay (ELISA) kit (*Hangzhou Eastbiopharm Co. Ltd.*, Hangzhou, China).

Children who had recovered from acute nasopharyngitis, acute otitis media, acute gastroenteritis, or seborrheic dermatitis were accepted as the control group. These children were admitted to our hospital during the same time period as the patient group. Subjects' parents or guardians provided written informed consent form. None of the subjects were using supplemental vitamin D. Children with renal disorders, epilepsy, malnutrition, obesity, diabetes mellitus, immune deficiency, or rickets were excluded from the study.

The data were analyzed using *SPSS version 20.0* (*SPSS Inc.*, Chicago, IL, USA). Descriptive analyses were expressed as percentages for categorical variables and mean ± one standard deviation (SD) for continuous variables. In the statistical evaluation, Chi-square test (for categorical variables), student T-test (for continuous variables), Mann-Whitney U test (for non-normally distributed variables), and correlation analyses were used. A P value < 0.05 was considered to be statistically significant. This study received ethical approval from the Ethics Committee of Bolu Abant İzzet Baysal University.

Results

Sixty-seven individuals, comprised of 43 upper UTI patients and 24 controls, were included in the study. Patients'ages ranged from 1-15 years, with a mean age of 6.9 (SD 4.2) years in the upper UTI group. Thirteen (30.2%) upper UTI patients were male and 30 (69.7%) were female. The groups were similar in terms of age, gender, and age group. The mean WBC, ANC, CRP, and ESR values of the upper UTI group were significantly higher than the those of the control group. Additionally, mean serum 25 (OH) D levels in upper UTI group were significantly lower than those in control group [18 (SD 9) *vs.* 23 (SD 10.6) ng/mL, respectively; P=0.045) (**Table 1**).

The upper UTI group was sub-divided into two groups: 22 (51.1%) with and 21 (48.8%) without renal parenchymal injury, according to the DMSA results. Both groups were similar in terms of mean age, age group, and gender. Laboratory findings of ANC, NLR, CRP, ESR, pyuria, and hematuria were

Table 1. Comparison of features of the upper UTI and control groups

Characteristics	Upper UTI (n=43)	Control (n=24)	P value
Mean age (SD), years	6.9 (4.2)	7.9 (3.8)	0.3*
Gender (F/M), n	30/13	13/11	0.2*
Age group (C/A), n	31/12	16/8	0.64*
Mean WBC (SD), /L	10,983 (3,206)	8,198 (2,271)	0.002
Mean ANC (SD), /L	6,229 (1,986)	4,283 (1,765)	0.001
Mean ALC (SD), /L	3,338 (1,873)	2,492 (658)	0.055
Mean NLR	2.4 (1.8)	1.8 (0.7)	0.14*
Mean CRP (SD), mg/dL	51.5 (48.8)	2.6 (2)	<0.001*
Mean ESR (SD), mm/h	39.5 (25)	8 (4.5)	0.01*
Mean 25 (OH) D (SD), ng/mL	18 (9)	23 (10.6)	0.045

*Mann-Whitney U; C: child; A: adolescent

also not significantly different between the two groups. Fourteen (32.5%) cases had been diagnosed with UTI for the first time, and 29 (67.4%) had recurrent UTIs. The majority of both groups had recurrent UTIs. We found that WBC was significantly higher in patients with than without renal parenchymal injury [12,065 (SD 3,359) *vs.* 9,630 (SD 2,518), respectively; P=0.041). In addition, 25(OH)D was significantly lower in patients with than without renal parenchymal injury [15.1 (SD 7.1) *vs.* 21 (SD 9.9) ng/mL, respectively; P=0.03] (**Table 2**).

The 22 patients with renal parenchymal injury were divided into two groups: 8 with acute renal parenchymal injury and 14 with RS, according to the DMSA findings. Mean WBC was significantly higher in patients with acute renal parenchymal injury than those with renal scarring [15,136 (SD 2332) *vs.* 10,530 (SD 2697), respectively; P=0.006]. However, mean 25(OH)D was not significantly lower in patients with acute renal parenchymal injury [12.5 (8.9) *vs.* 16.6 (5.7) ng/ml, respectively; P=0.14]. The other features were also not significantly different between two groups (**Table 3**).

Fifteen of the renal parenchymal injury patients were diagnosed with recurrent UTI. Mean 25(OH)D was lower in patients with recurrent UTI than in those with first UTI, but the difference was not significant [17.4 (10) *vs.* 19.2 (6.7) ng/mL, respectively; P=0.53]. Twelve of the renal parenchymal injury patients had VUR, but there was no significant difference in mean 25(OH)D between those with and without VUR [14.6 (6.2) *vs.* 20.9 (10.7) ng/mL, respectively; P=0.057]. On the other hand mean 25(OH)D was significantly lower in patients with acute renal parenchymal injury

Table 2. Comparison of upper UTI patıents with and without renal parenchymal injury

Variables	With renal parenchymal injury (n=22)	Without renal parenchymal injury (n=21)	P value
Mean age (SD), years	6.9 (4.3)	6.9 (4.1)	0.97*
Gender, F/M, n	17/5	13/8	0.27*
Age group, C/A, n	15/7	16/5	0.92*
Mean WBC (SD), /L	12,065 (3,359)	9,630 (2,518)	0.041
Mean ANC (SD), /L	6,854 (1,839)	5,447 (1,953)	0.066
Mean ALC (SD), /L	3,338 (1,442)	3,339 (2,377)	0.32*
Mean NLR (SD)	2.3 (0.9)	2.6 (2.6)	0.79*
Mean CRP (SD), mg/dL	58.8 (63.9)	40.7 (8.9)	0.76*
Mean ESR (SD), mm/h	53 (31.7)	26 (2.6)	0.4*
Pyuria, n	14	10	0.29*
Hematuria, n	10	6	0.25*
VUR, n/total	12/21	3/11	0.18*
Recurrent UTI, %	68.1	66.6	0.91*
Mean 25 (OH) D (SD), ng/mL	15.1 (7.1)	21 (9.9)	0.03

*Mann-Whitney U; VUR: vesicoureteral reflux)

than the those of the control group [12.5 (8.9) *vs.* 23 (10.6) ng/mL, respectively; P=0.018]. In addition, mean 25(OH)D was also significantly lower in patients with renal scarring than the those of the control group [16.6 (5.7) *vs.* 23 (10.6) ng/ml, respectively; P=0.045] (**Table 4**).

Table 3. Comparison of features of renal parenchymal injury types

Variables	Acute renal parenchymal injury (n=8)	Renal scarring (n=14)	P value
Mean age (SD), years	6 (4.1)	7.5 (4.4)	0.48*
Gender, F/M, n	6/2	11/3	0.92*
Age group, C/A, n	7/1	9/5	0.4*
Mean WBC (SD), /L	15,136 (2,332)	10,530 (2,697)	0.006
Mean ANC (SD), /L	7,378 (852)	6,593 (2,171)	0.45
Mean ALC (SD), /L	2,956 (1,189)	3,529 (1,576)	0.48
Mean NLR (SD)	2.8 (1.1)	2 (0.7)	0.17
Mean CRP (SD), mg/dL	65.5 (69)	25 (??)	0.66*
Mean ESR (SD), mm/h	36 (16.9)	87 (??)	0.99*
Pyuria, n	5	9	0.99*
Hematuria, n	4	6	0.81*
VUR, n/total	4/7	8/14	0.98*
Recurrent UTI, %	75	64.2	0.71*
Mean 25 (OH) D (SD), ng/mL	12.5 (8.9)	16.6 (5.7)	0.14*

*Mann-Whitney U

Table 4. Comparison of 25 (OH) D levels of renal parenchymal injury types and control group.

Variables	Acute renal parenchymal injury (n=8)	Renal scarring (n=14)	Control (n=24)	P value
Mean 25(OH)D (SD), ng/mL	12.5 (8.9)[a]	16.6 (5.7)[b]	23 (10.6)[c]	0.018[a,c] 0.045[b,c]

Discussion

Vitamin D deficiency is a major, worldwide, public health problem. It is associated with cancer, autoimmune disease, and cardiovascular disease.[18] Vitamin D deficiency causes dysregulation in the immune system. In particular, changes in cathelicidins are associated with infection. Cathelicidins attracts cell such as dendritic cells, T cells, monocytes, macrophages, and neutrophils to sites of inflammation.[19,20] Therefore, they are considered to be an important component of the innate immune system with a role in inflammation.[21,22] Abundant amounts of cathelicidins in neutrophils have antibacterial (against both Gram-positive and -negative), antifungal, and antiviral effects as they act to destroy cell membrane integrity.[23-25] Since vitamin D receptors are present on dendritic cells, T cells, monocytes, and neutrophils,[20] there is likely a relationship between vitamin D and cathelicidins. Vitamin D deficiency causes an increase in infections as cathelicidins decrease.[18] An UTI is an inflammatory response that occurs as a result of bacterial adhesion to the uroepithelium. Leukocytes, dendritic cells, macrophages, and other cells with cathelicidins move to the affected renal parenchymal area during UTIs.[26] The UTIs can worsen due to impaired function of these cells which have vitamin D receptors. We found a significant relationship between decreased serum vitamin D and upper UTIs, in agreement with another study.[11] Past studies have noted a relationship between vitamin D deficiency and recurrent respiratory infections.[27,28] However, there are few publications on the relationship between recurrent UTIs and decreased vitamin D level.[29] We did not find such a relationship in our study.

Vitamin D deficiency has been associated with upper UTIs.[11] Granulocytes and released bacterial toxins together cause parenchymal injury through

capillary obstruction and hypoxia. Free oxygen radicals released in the hypoperfusion-reperfusion cycle are considered to be the reason for post-infectious renal parenchymal injury.[30] Inflammatory cells, in particular, granulocytes due to the enzymes they contain, are responsible for renal parenchymal injury.[31] Vitamin D was found to be effective on apoptosis, apart from the inflammatory response. Vitamin D has also been shown to protect kidney function by inhibiting apoptosis,[32] and vitamin D deficiency causes increased apoptosis of renal tubular cells.[33] Vitamin D deficiency leads to a decrease in megalin, which is a receptor for 25(OH)D–D-binding protein in renal proximal tubules,[32] thus increasing the effects of the disease even further. Our study showed that renal parenchymal injury may be associated with decreased serum vitamin D levels. Also, serum vitamin D levels were lower in renal parenchymal injury types when compared to the healthy subjects. However we couldn't find a difference in serum vitamin D levels between renal parenchymal injury types. We think the small number of subjects may have caused this result.

Other than bacterial virulence factors, severity of inflammation is the most important factor affecting renal parenchymal injury. The most important indicator of this severity is the increase in acute phase reactants.[34] The NLR is also used as a diagnostic marker of upper UTI.[35] We demonstrated the severity of inflammation with high WBC in patients who had acute renal parenchymal injury. However, the other markers were not significantly different in these patients.

Renal scarring is a result of renal parenchymal inflammation. Bacterial infections activate phagocytotic neutrophils and macrophages. Phagocytosis leads to the release of cytokines, arachidonic acid, oxygen radicals, and lysosomal enzymes. This process, while intending to destroy bacteria, also results in tissue injury and eventually scar formation.[36] Macrophages induce fibrosis through activation and proliferation of fibroblasts.[37] Vitamin D is known to have significant effects on collagen synthesis and degradation; as such, it may have anti-proliferative effects, especially in fibroproliferative diseases. These effects are displayed via transforming growth factor-β1 and hepatocyte growth factor. In vitamin D deficiency, suppression of the transforming growth factor-β1 gene, which is effective for fibrosis, is eliminated, whereas up-regulation of the anti-fibrotic hepatocyte growth factor gene is suppressed.[38] Hence, fibroblasts are effective in scar formation. Although not statistically significant, we found that vitamin D was low in subjects with RS. The small number of patients and short half-life of 25(OH)D may have affected this result. Many factors may change serum vitamin D levels within six-month periods. As such, low vitamin D may facilitate the occurrence of RS. In addition, a study reported that VUR and recurrent UTIs are risk factors for RS following UTIs.[39] However we couldn't find a relationship between the vitamin D levels and recurrent UTI or VUR.

The main limitations of our study were the limited sample size, subjects from only a single center, and not investigating the roles of vitamin D metabolites, vitamin D receptors, or cathelicidin, as well as whether vitamin D replacement prevented RS.

In conclusion, decreased vitamin D is associated with upper UTIs and renal parenchymal injury. Vitamin D is lower in patients with renal scarring compared with acute renal parenchymal injury, but it is not statistically significant. More studies are needed to determine whether decreased vitamin D is a risk factor for renal scarring.

Conflict of interest

None declared.

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Original Article

Duration of active epilepsy as a predictor of seizure control after relapse in child epilepsy

Charles Subakti, Madarina Julia, Agung Triono

Abstract

Background Epilepsy is a chronic illness that may affect childhood growth and development. Some epilepsy cases are easy to control, either with monotherapy or politherapy antiepileptic-drugs, but many cases are difficult to control. Several factors influence the risk of relapse, but information is limited on factors predictive of seizure control after relapse. Our study investigate patient with epilepsy relaps and see whether the duration of active epilepsy prior to initial remission can be use as a predictor of seizure control after relaps.

Objective To assess whether duration of active epilepsy was predictive of seizure control after relapse.

Methods This retrospective cohort study was performed in Dr Sardjito Hospital, Yogyakarta, on epileptic relapse patients aged 2 to 18 years , who had achieved remission for at least a 2-year seizure-free interval, and relapsed after antiepileptic drud (AED) discontinuation. We excluded patients with progressive neurological diseases, inborn errors of metabolism, febrile seizures, and those who could not be followed up for at least 2 years, or those with incomplete medical records. Subjects were divided into those who had a duration of active epilepsy prior to initial remission within 6 months and ≥6 months. Time to seizure control after relapse was analysed by Kaplan-Meier survival analysis.

Results A total of 80 patients were included in the study. Overall median for seizure control after relapse was 3.6 (95%CI 1.1 to 6.0) months. Median for seizure control after relapse for those who had a duration of active epilepsy prior to initial remission within 6 months and ≥ 6 months were 3 (95%CI 0.1 to 5.8) months and 12 (95%CI 4.4 to 19.5) months, respectively. Log-rank test revealed no significant difference between groups (P=0.12).

Conclusion Duration of active epilepsy prior to initial remission was not a predictor for seizure control after relapse. **[Paediatr Indones. 2020;60:212-7 ; DOI: 10.14238/pi60.4.2020.212-7]**.

Keywords: *active epilepsy; predictor; seizure control; relapse*

Epilepsy is a chronic disease with the potential to disturb child development. Survival rates of infants at high risk of neurological and developmental disorders, including epilepsy, have increased in developing countries like Indonesia, because of increased availability of medical services for perinatal and infant patients.[1-3] Epilepsy is a neurologic condition characterized by complex symptoms such as irregular and excessive sudden paroxysmal nerve excitation caused by various pathological processes in the brain.[4] The incidence of epilepsy in children is higher than in adults, and usually starts at a young age. In developing countries, the incidence of epilepsy in children is around 40 cases/100,000 children per year.[2,3,5]

Approximately two-thirds of children with epilepsy reach a seizure-free state within the first or second anti-epileptic drug (AED) therapy,

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while the rest (around 35%) have intractable or difficult to control conditions.[6,7] Various factors should be considered in the withdrawal of AEDs, based on individual assessments including AED effects during the first therapy, side effects, psychosocial considerations, and if seizure relapse occurs within the withdrawal period.[4] Inappropriate AED withdrawal increases the risk of relapse in controlled epilepsy. If relapse occurs during AED withdrawal, complete remission of epilepsy is more difficult to achieve. In fact, approximately 63% of patients who relapse develop drug resistance.[8] Discontinuation of AEDs should be considered for children who have been seizure-free for at least 2 consecutive years, as 60-70% of these children do not experience relapse after stopping the AEDs. For the remainder who experience relapse, long-term remission can still be achieved by standard treatment protocols.[3]

Several factors reportedly can be used to predict relapse: symptomatic epilepsy, epilepsy syndrome, age of onset, seizure-free interval duration, and abnormal EEG imaging if AEDs are discontinued.[3] Relapse risk after AED discontinuation in children was estimated to be 16-56%.8 Other systematic reviews, including 28 case reports involving 2,758 children with most having a seizure-free interval of more than 2 years before AED withdrawal, concluded that cumulative probability for being seizure-free after AED withdrawal was around 66-96% in the first year, and 61-91% in the second year. Furthermore, relapse risk was higher in the first year (especially first six months) after stopping AEDs, and then decreased in the following months.[8,9]

To our knowledge, this is the first study on predictive factors of seizure control after relapse in Indonesia. We aimed to assess if the duration of active epilepsy before AED therapy was a predictor for the time to seizure control after relapse.

Methods

This retrospective study was done in Dr. Sardjito Hospital, Yogyakarta, using medical record data. The study period was January 2012 to December 2017, and subjects were included by purposive sampling. All medical records were assessed by a pediatric neurologist to determine if patients met the inclusion criteria, which were children aged 2-18 years, diagnosed with epilepsy, had achieved remission for at least a 2-year seizure-free interval, and relapsed after AED discontinuation. Patients with progressive neurological diseases, inborn errors of metabolism, febrile seizures, those who could not be followed up for at least 2 years, or those with incomplete medical record data were excluded. A total of 80 subjects were divided into two groups of 40 subjects each: those with duration of active epilepsy prior to initial remission was less than 6 months , and those with duration of active epilepsy prior to initial remission was >6 months.

Data were analyzed using the *Statistical Package for the Social Science (SPSS) version 20.0* software. Chi-square analysis was used for bivariate data, followed by Mantel-Haenzel test for multivariate analysis of confounding variables. Survival analysis was done to assess time-to-event outcomes. Ethical approval of the study was obtained from the Ethics Committee of the Universitas Gadjah Mada Medical School, Indonesia.

Results

Baseline characteristics of study subjects are shown in **Table 1**. The ratio between males and females was 1:1. The median patient age at onset of epilepsy diagnosis was 6.04 (range 0.08-16) years, and 51.2% of patients were >6 years old at diagnosis. The median age at the time of relapse was 9.49 (range 2-18) years. Most subjects relapsed in the first six months after AED withdrawal (**Table 2**).

Bivariate analysis results of potentially predictive factors in seizure control post-relapse are shown in **Table 2**. Using risk ratio calculation, duration of active epilepsy prior to initial remission did not influence the seizure control after relapse (RR 1.42; 95%CI 0.55 to 3.67; P=0.47).

Analysis was continued using a Kaplan-Meier curve. From a total of 80 subjects, overall median time to seizure control after relapse was 3.6 (95%CI 1.1 to 6.0) months (**Figure 1**). The median time to seizure control after relapse in the group with < 6 month duration of active epilepsy prior to initial remission was 3 (95%CI 0.1 to 5.8) months, while that of the group with ≥ 6 month duration of active epilepsy was 12 (95%CI 4.4 to 19.5) months. Log-rank test revealed no significant differences between the two groups (P=0.12).

Table 1. Baseline characteristics of subjects

Characteristics	N=80
Gender	
Male, n (%)	40 (50)
Female, n (%)	40 (50)
Age at onset of epilepsy diagnosis, n (%)	
< 6 years	39 (48.8)
≥ 6 years	41 (51.2)
Median age at onset of epilepsy diagnosis (range), years	6.04 (0.08-16)
Median age at the time of relapse diagnosis (range), years	9.49 (2-18)
Time to relapse after AED withdrawal	
< 6 months	48 (60)
6-12 months	32 (40)
Seizure type, n (%)	
Partial	19 (23.8)
General	51 (76.2)
Seizure etiology, n (%)	
Symptomatic	41 (51.2)
Idiopathic	39 (48.8)
AED therapy, n (%)	
Polytherapy	11 (13.8)
Monotherapy	69 (86.2)
EEG evaluation, n (%)	
Epileptiform	67 (83.8)
Normal	13 (16.2)
History of status epilepticus, n (%)	
Yes	16 (20)
No	64 (80)
History of epilepsy in the family, n (%)	
Yes	13 (16.2)
No	67 (83.8)
Head CT-scan results, n (%)*	
Abnormal	21 (91.3)
Normal	2 (8.7)
Seizure frequency before AED withdrawal, n (%)	46 (57.5)
> 1x/month	34 (42.5)
< 1x/month	
Seizure control after relapse	
< 6 months	50 (62.5)
6-12 months	12 (15)
1-2 years	9 (11.2)
>2 years	9 (11.2)

*CT scan was only performed in 23 subjects

Discussion

The relapse rate in the last year of our observation was 23 (6.2%), as shown in a study about the predictive factors of relapse in children with epilepsy after AED withdrawal in Dr. Sardjito Hospital, Yogyakarta, showed the prevalence of relapse was 3.6%.10 Reported rates varied from 22.4% to 40% in other studies.[6,10,11] This discrepancy may be due to the heterogeneity of the population, as well as different criteria for relapse, follow-up periods, and statistical methods. The relapse criteria in our study were patients who had achieved remission (seizure-free) for at least 2 consecutive years and experienced seizures at the AED withdrawal phase or after AED discontinuation. Our relatively lower relapse rate may have been due to our exclusion of patients with progressive neurological deficits or inborn errors of metabolism, which have poor prognoses and have higher relapse rates. In addition, the low incidence of relapse in our study may have also been due to the predominance of the general seizure type, the use of monotherapy, and few subjects with a history of status epilepticus. Similarly, a previous study showed that relapse could be predicted by abnormal EEGs (especially epileptiform), onset of epilepsy (higher risk if <2 years, or >10 years of age), history of status epilepticus, intellectual disability, and seizure frequencies before and during AED treatment.[5]

Few investigators have addressed the question of what happens to children when medication is withdrawn after prolonged seizure control, but no study has specifically investigated the after relapse condition. Some authors found that females had a higher rate of relapse. A study showed that females predominated the relapse group (52.4% females vs. 47.6% males), but the difference was not statistically significant.10 In our study with 40 female subjects, 70% (28/40) attained seizure control at < 6 months after relapse.

Overall median age at epilepsy diagnosis in our study was 6.04 (range 0.08-16) years. From total subjects that had first relapse episode, 41 (51.2%) subjects had the onset of epilepsy at ≥ 6 year old compared to those diagnosed onset of epilepsy at < 6 years of age. In contrast, a previous study showed that children diagnosed with epilepsy at >6 years of age had a 29.4% greater risk of relapse compared to those diagnosed with epilepsy at <6 years of age.[9] This discrepancy may support other study that showed age was not a predictor for seizure relapse in epilepsy.

In our study, 62.5% of subjects needed less than 6 months to seizure control after relapse, the relapse

Table 2. Bivariate analysis of potential factors predictive of seizure control after relapse

	Seizure control after relapse				
Variables	≥ 6 months (n=25)	<6 months (n=55)	RR	95%CI	P value
Duration of active epilepsy, n					
≥ 6 months	14	26	1.42	0.54 to 367	0.46
< 6 months	11	29			
Seizure type, n					
Partial	6	13	1.02	0.33 to 3.09	0.97
General	19	42			
Seizure etiology, n					
Symptomatic	15	26	1.67	0.64 to 4.36	0.29
Idiopathic	10	29			
AED therapy, n					
Polytherapy	6	5	3.15	0 .86 to 11.5	0.09
Monotherapy	19	50			
EEG evaluation, n					
Epileptiform	22	45	1.63	0.41 to 6.52	0.74
Normal	3	10			

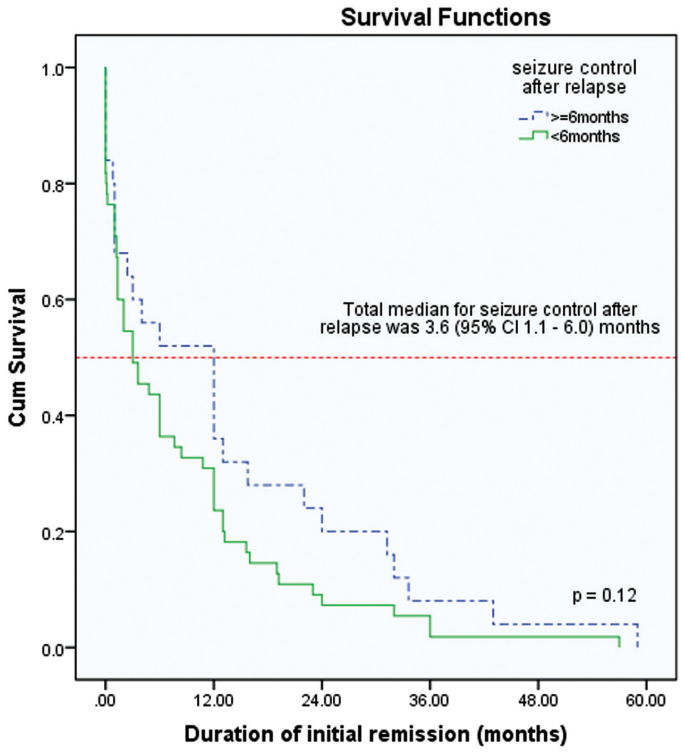

Figure 1. Kaplan-Meier curve of time to seizure control based on duration of active epilepsy groups

controlled in the first year was 15%, and after the second year at 11.2%. Similarly, a study noted that 65.3% of patients needed <6 months to control their seizure after relapse, and 34.7% patients need ≥ 6 months to control their seizure after relapse.[10] But in our study, the duration of active epilepsy prior to initial remission was not significantly related to seizure control after relapse.

Bivariate analysis showed no significant differences between groups with seizure control time <6 months and ≥6 months, with regards to seizure type, seizure etiology, type of AED treatment, or EEG evaluation at AED withdrawal. The duration of active epilepsy was not significantly different between the two groups of seizure control duration after relapse (RR 1.42; 95%CI 0.55 to 3.67; P=0.47). In our study, monotherapy had RR of 3.15 (95%CI 0.86 to 11.5; P=0.09) compared to polytherapy. Despite the predominance of subjects using monotherapy (86.2% *vs.* 13.8%, respectively), therapy type may be a potential predictor for seizure control duration after relapse. Further investigation that compares closer to equal numbers of monotherapy vs. polytherapy is needed. In addition, general seizure type was dominant in our study (76.2%) and is more likely to have a positive response to AED treatment. This result may explain why the majority of our subjects had seizure control after relapse of < 6 months (68.8%).

Survival analysis showed that for the 80 patients in this study, the total median for seizure control after relapse was 3.6 (95%CI 1.1 to 6.0) months. In the <6 month duration of active epilepsy group, the median for seizure control was 3.0 (95%CI 0.1 to 5.8) months, while that of the group with ≥6 month duration of active epilepsy was 12.0 (95%CI 4.4 to 19.5) months. Log-rank test revealed no statistically significant relationship between duration of active epilepsy prior to initial remission and seizure control duration after relapse (P=0.12). Although there was no direct correlation between duration of active epilepsy and seizure control time in relapse patients, the shorter duration of active epilepsy tended to indicate the sooner the relapse would be controlled.

The main limitation of our study was the retrospective retrieval of information from medical records, which may have led to information bias. To our knowledge, there were no studies that investigated the clinical conditions after relapse in epilepsy patients and factors that can be used as positive or negative predictors. In conclusion, the duration of active epilepsy prior to initial remission cannot be used as a predictor for seizure control duration after relapse. As such, we recommend a future study with a prospective design, more homogeneous subjects, longer follow-up time, and analysis of other potential predictive factors of relapse after AED withdrawal in epileptic children.

Conflict of Interest

None declared.

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Original Article

Use of Xpert MTB/RIF for diagnosis of pediatric tuberculosis in Indonesia

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Abstract

Background The Xpert MTB/RIF assay demonstrated a better diagnostic value than sputum smear for TB in adults and children.

Objective To evaluate the use of Xpert MTB/RIF for TB diagnosis in children.

Methods We conducted a prospective study in Yogyakarta, Indonesia, involving 19 primary health centers (PHCs) and one provincial hospital. Children aged 0-14 years with suspected TB who visited the study sites were screened. Subjects underwent history-taking, physical examination, tuberculin skin test, chest X-ray, as well as sputum induction for Xpert MTB/RIF assay, sputum smear, and TB culture. The diagnosis of TB was made by doctors based on the results of investigations, as follows: certain TB (bacteriological confirmation), probable TB, and possible TB.

Results Of 80 subjects, 21 (26%) were diagnosed with TB disease (4 certain TB and 17 probable TB). Sputum induction was successfully performed in 79 children. None of the children had positive sputum smears. Mycobacterium tuberculosis was detected by Xpert MTB/RIF in 4 children, accounting for 5% of all children with suspected TB, or 19% among children with TB disease. The 4 Xpert MTB/RIF-positive subjects had severe TB disease and were rifampicin-sensitive.

Conclusion Xpert MTB/RIF may improve case finding among children with severe TB disease with negative sputum smear. **[Paediatr Indones. 2020;60:198-204; DOI: 10.14238/pi60.4.2020.198-204]**.

Keywords: *Xpert MTB/RIF; tuberculosis; children*

Identification of Mycobacterium tuberculosis in respiratory specimens is regarded as the gold standard of pulmonary TB diagnosis. While it is now the most important diagnostic approach in the era of increasing multidrug resistant TB, including in children, challenges remain due to difficulties in obtaining sputum specimens and the paucibacillary nature of TB in children.[1] The Xpert MTB/RIF assay, an automated molecular test using real-time polymerase chain reaction, provides several benefits such as simultaneously detecting the presence of Mycobacterium tuberculosis DNA and rifampicin sensitivity in less than 2 hours, and improved diagnostic value. The specificity of Xpert MTB/RIF compared to a clinical reference standard was at least 99%. In comparison to sputum smear, the Xpert MTB/RIF sensitivity was 37% higher on expectorated or induced sputum and 44% higher on gastric lavage

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specimens.[2,3] Despite low quality evidence, in 2014 the WHO recommended the use of Xpert MTB/RIF as the initial diagnostic test for pediatric TB.[3]

Indonesia ranked 3rd among the 22 countries with the highest TB burden, with an estimated 322,806 TB cases in 2014, which contributed 10% of total global cases.[4] Of these, only 7% of cases were under 15 years of age. The problem of underdiagnosis of pediatric TB has been acknowledged worldwide. Underdiagnosis is mainly observed at the primary health care level, which has limited resources for diagnosing TB. On the contrary, in urban areas of Indonesia pediatric TB tends to be overdiagnosed but underreported by doctors in private practice.[5,6] In line with the WHO recommendation and to improve case finding and diagnosis quality in children, the Indonesia National TB program recommended the use of Xpert MTB/RIF in children in 2015. We aimed to evaluate small-scale implementation of Xpert MTB/RIF for children in Yogyakarta, Indonesia, including the use of sputum induction method to collect sputum specimens in children.

Methods

We conducted a prospective study in Yogyakarta municipality from March to October 2015 in 19 primary health centers (PHCs). The PHC health workers were informed about a new recommendation to use Xpert MTB/RIF for pediatric TB. They were asked to screen children aged 0-14 years who visited the PHCs with TB symptoms or close contact to an infectious TB case, and to refer them to Dr. Sardjito Hospital, a provincial hospital, for further TB investigations. Tuberculosis symptoms included persistent cough (cough for 2 weeks or more that did not improve with antibiotics or anti-asthma treatment), fever (body temperature > 38°C for 2 weeks or more after common causes such as typhoid, urinary tract infection, or malaria had been excluded), and weight loss or failure to thrive. Screening of children with suspected TB was also done in the Department of Pediatrics Outpatient Clinic and Ward, Dr. Sarjito Hospital by a study pediatrician. Children suspected to have extrapulmonary TB (meningitis TB, miliary TB, etc.) were also screened by a study pediatrician and recruited into the study.

Subjects underwent history-taking, physical examination, nutritional assessment, tuberculin skin test (TST), chest X-ray (CXR), and sputum induction in the hospital. The sputum was collected for Xpert/MTB RIF assay, smear, and solid culture for Mycobacterium tuberculosis. A pediatrician collected cerebrospinal fluid from a child who was suspected to have TB meningitis. A trained study nurse performed TST by intradermally injecting 0.1 mL of 2 TU tuberculin purified protein derivate RT 23 in the volar aspect of the forearm. The TST results were assessed by measuring the transverse induration diameter at 72 hours, and were considered positive if the diameter was > 10 mm, regardless of BCG vaccination status. The CXR was performed in both antero-posterior and lateral views, and was interpreted by a radiologist and a pediatrician who were blinded to subjects' clinical information. In case of disagreement, a consensus was made by both radiologist and pediatrician.

A trained nurse attempted to collect sputum at least once using the sputum induction method. A second sputum specimen was obtained on the same day, a minimum of 4 hours after the first specimen was obtained. The following procedure was done according to standard protocol after 2-3 hours of fasting: the child received 200 ug salbutamol via jet nebulizer for 20 minutes to prevent bronchoconstriction, followed by 5 mL of 3% sterile saline via jet nebulizer for 20 minutes. The nurse then performed chest percussion over the anterior and posterior chest wall. If the child could not expectorate sputum unassisted, sputum was obtained by suctioning through the nasopharynx with a sterile mucus extractor of catheter size 6 or 7 as the child was coughing. The procedure was performed with continuous monitoring of pulse and oxygen saturation. If oxygen saturation fell below 92% then the procedure was halted. The sputum trap was sealed and the specimen was transported directly to the Microbiology Laboratory. A laboratory technician assessed the sputum macroscopically for quality. Good quality sputum was considered to have adequate volume (2 mL), the presence of mucoid or mucopurulent material, and no obvious food particles or other solid particulate in the sputum. Poor quality specimens were thin and watery (saliva). Another specimen (cerebrospinal fluid) was collected from a child with suspected TB meningitis.

The specimens were tested for acid fast bacilli (AFB), cultured for mycobacteria on Lowenstein-Jensen media, and used for Xpert MTB/RIF assay (*Cepheid,* Sunnyvale, California). The specimens were processed for Xpert MTB/RIF as follows: 1 mL of uncentrifuged specimen was mixed with the sample reagent (*Cepheid*) in a 1:2 ratio. The mixture was shaken and allowed to stand for 10 minutes, then shaken again and allowed to stand for another 5 minutes. Two mL of the mixture was put into an Xpert cartridge and inserted into the Xpert MTB/RIF machine (*Cepheid Inc.*). The result was obtained within 2 hours.

A study pediatrician established the diagnosis of TB disease based on the following criteria: certain TB (bacteriologic confirmation for Mycobacterium tuberculosis, either from the positive result of sputum smear, Xpert MTB/RIF, of sputum culture); probable TB (had at least one TB symptom, and CXR was consistent with intrathoracic TB or there was supportive evidence of extrapulmonary TB; and there was a positive clinical response to anti TB treatment); possible TB (had at least one TB symptom and either of the following: a positive clinical response to anti-TB treatment or CXR was consistent with intrathoracic TB). Latent TB infection was diagnosed in children with positive TST result in the absence of TB disease. Ethical approval of the study was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada, Indonesia. Informed consent was obtained from parents/guardians prior to the procedures. The data were analyzed descriptively as proportion or frequency for categorical variables and as mean or median for continuous variables. Statistical analysis was performed using *STATA software version 12* (*StataCorp*, College Station, Texas).

Results

A total of 106 children were screened and suspected as having TB disease in the PHCs (78 children) and the hospital (28 children) during the study period. Twenty children screened in the PHCs and referred did not visit the hospital due to parent refusal (12 children), having no time to visit the hospital during working hours (7 children), or further TB investigation being done in a private hospital (1 child). Six children from the PHCs were excluded because their symptoms were not consistent with TB (**Figure 1**). Hence, 80 children were included; their characteristics are presented in **Table 1**. Sixty (75%) children had TB exposure with 29 reporting contacts, 5 children had positive TST, and 26 had both contact and positive TST. Of those

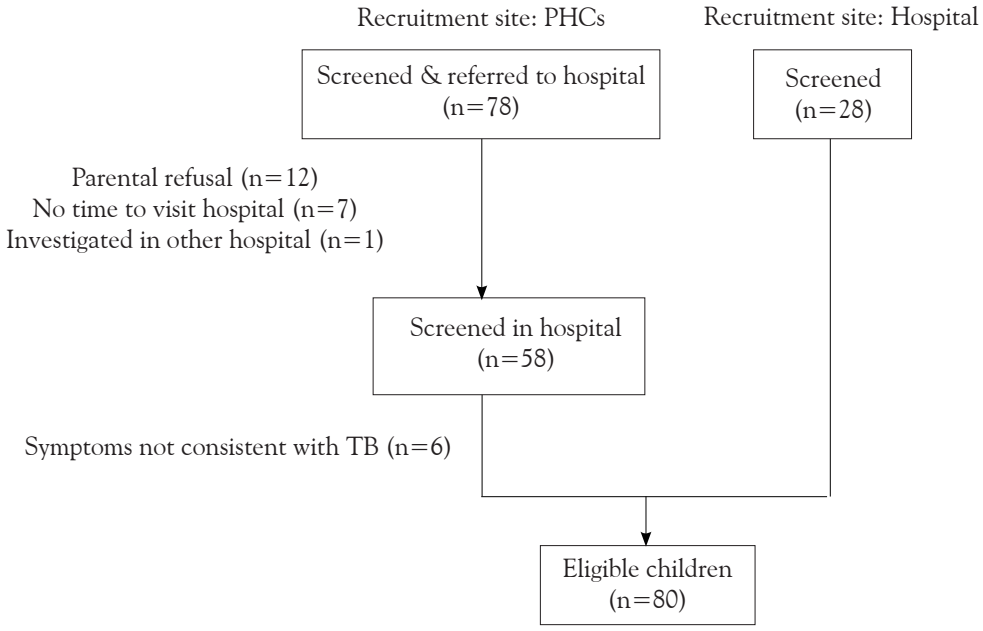

Figure 1. Screening of children with suspected TB

Table 1. Characteristics of subjects

Characteristics	(N = 80)
Male sex, n (%)	50 (62.5)
Median age, years (IQR)	4.4 (1.9; 8.5)
Age < 5 years, n (%)	43 (53.8)
Nutritional status, n (%)	
Well-nourished	43 (53.7)
Malnourished	30 (37.5)
Severely malnourished	7 (8.8)
Referral from PHC, n (%)	52 (65)
BCG vaccination, n (%)	79 (99)
Close contact with a TB case, n (%)	55 (68.8)
HIV (+), n (%)	2 (2.5)

with contact, 9 were multidrugs resistant (MDR) TB contacts. Two children had been diagnosed with HIV infection before screening for TB. HIV status of the other subjects was unknown, as HIV test was not performed in this study. Almost all children had received BCG vaccination, but BCG status was unknown for one child (no BCG scar, no immunization record, and the parent did not remember).

Of 80 subjects, 67 (83.3%) had TB symptoms. The 13 children without TB symptoms were included due to their past close contact with a TB case. Most children presented with symptoms of fever, cough, and weight loss. Other symptoms documented were seizure (1 child), stridor (1 child), and dyspnea (5 children). Tuberculin skin test was positive in 31 (38.8%) children. Miliary pattern on CXR was identified in 2 children, whereas pleural effusion and consolidation were identified in one child each.

One hundred thirty-two sputum specimens were collected from 79 children and one cerebrospinal specimen was collected from one child. Fifty-three children provided two sputum specimens each, whereas 26 children only provided one specimen each. The most common reason for not collecting the second sputum was parent refusal. Adverse event of epistaxis occurred in 2 children. Macroscopic assessment of sputum specimens revealed that only 45 (34.1%) specimens were good quality. None of the specimens showed positive smear results.

Mycobacterium tuberculosis was detected by Xpert MTB/RIF assay in 4 (5%) subjects (in three sputum specimens and one cerebrospinal fluid specimen). This accounted for 6% of the 67 children with TB symptoms or 19% of 21 children with TB disease. Among good quality sputum specimens, the proportion of positive Xpert MTB/RIF was 6.7% (3/45). None was resistant to rifampicin. All of the positive Xpert/MTB RIF subjects had negative sputum smears. Solid culture of *Mycobacterium tuberculosis* was grown in one subject. None of the asymptomatic subjects had positive results of microbiological investigations.

Based on the investigation results, 21 (26%) children were diagnosed with TB disease (4 certain TB and 17 probable TB) and 14 (17%) children with latent TB infection. Of 21 children with TB disease, 5 had severe TB, 2 had miliary TB, 1 had TB meningitis, 1 had TB HIV, and 1 had pleural effusion. The characteristics and investigation results of the 4 children with positive Xpert MTB/RIF are shown in **Table 2**. None of the children recruited from the PHCs had positive Xpert MTB/RIF results. The 4 children with positive Xpert MTB/RIF results had severe TB and the following conditions: TB-HIV (1), TB meningitis (1), miliary TB (1), and laryngeal and miliary TB (1).

Discussion

Our study shows that Xpert MTB/RIF assay identified more TB cases than sputum smears. None of the

Table 2. Characteristics of the 4 subjects with positive Xpert MTB/RIF results

No	Gender	Age	Site	Symptoms	Close contact	CXR	TST, mm	Culture	HIV
1	M	9 yrs	Hospital	Cough, fever, dyspnea	Yes	Consolidation	0	No growth	Positive
2	F	7 mo	Hospital	Fever, cough, stridor, weight loss	Yes	Miliary pattern	7	No growth	Not tested
3	M	14	Hospital	Fever, seizures	Yes	Hilar	0	Growth	Not tested
4	M	7 mo	Hospital	Fever, cough	Yes	Miliary pattern	0	No growth	Not tested

smear specimens was positive for TB, whereas Xpert MTB/RIF was positive in 5% of children who were suspected to have TB. Among symptomatic children, the proportion was 6%, and increased to 19% in children with TB disease. A meta-analysis of 15 studies on the use of Xpert MTB/RIF in children reported an average positive result of 11%, which ranged between 1% in Malawi to 45% in Vietnam.[2] Xpert MTB/RIF has 35-44% higher sensitivity compared to sputum smears. Compared to culture, the sensitivity of Xpert MTB/RIF ranged from 25% to 100%, with a pooled sensitivity of 62% and specificity of 93%-100%, with a pooled sensitivity of 98%.[2] Our study was not designed to determine sensitivity and specificity of Xpert MTB/RIF, but if it is compared to culture as the gold standard, the sensitivity of Xpert MTB/RIF was 100% with a specificity of 98%.

The majority of studies on Xpert MTB/RIF in children were hospital- based studies that involved inpatient children.[7-11] One study from South Africa included outpatients from a primary care setting and documented positive Xpert MTB/RIF results in 7% of 384 children.[12] Our study recruited children from both community (PHCs) and hospital (both inpatient and outpatient) settings, but the positive Xpert MTB/RIF results were only identified in children who were hospitalized with severe TB. Higher proportions of positive Xpert MTB/RIF among hospitalized children compared to outpatients were shown by a stratified analysis of a meta-analysis.[2] Hospitalized children often have severe TB with higher bacterial loads which are more likely to have positive smears and cultures.[13] The performance of Xpert MTB/RIF was associated with smear status, in which sensitivity of Xpert MTB/RIF was greater in individuals who had higher mycobacterial load.[3,14] Xpert MTB/RIF detected 96% of smear-positive children from samples of expectorated or induced sputum, compared to 55% of smear-negative culture positive children with the same specimen.[3] The role of Xpert MTB/RIF for TB diagnosis among child contacts has also been evaluated.[15] The sensitivity of Xpert MTB/RIF was higher than sputum smear. Nevertheless, compared to hospital-based studies the sensitivity of Xpert MTB/RIF compared to culture in the context of contact investigation was lower.

Sputum induction was initially used in the late 1980s to collect sputum specimen in HIV-infected patients to identify *Pneumocystis jirroveci*.[16] In the last decade, this method was recommended to collect sputum in children for TB diagnosis and has been shown to be safe, feasible, and improve case finding.[17-19] This method is simpler than gastric lavage and can be performed in an outpatient clinic. Furthermore, a previous study reported that one sputum specimen from sputum induction was as effective as two sputum specimens from gastric lavage.[20] In Indonesia this method has not been routinely performed. Sputum induction in our study was conducted in the hospital by trained nurses. In general, it was feasible for sputum induction to be performed in our study, with only 2 children experiencing epistaxis. Nevertheless, only 34.1% of sputum specimens were good quality. This could have been due to an improper sputum collection technique, an uncooperative child, or the child not able to produce sputum. Most children in this study were aged less than 5 years, and 50% of them were less than 2 years of age. In addition, most children in this study had mild symptoms, in whom sputum production was minimal or absent.

Bacteriological confirmation for the diagnosis of TB in children is now recommended by the WHO.[21] Not only does it improve the quality of diagnosis, but also is important in the era of increasing cases of multidrug resistant TB. The majority of previous studies, including our study, processed the specimens for bacteriological test at university hospital laboratories. Nevertheless, sputum collection should be able to be done at the primary health care level, making hospital referral unnecessary. Our study documented that a quarter of children from PHCs did not come to the hospital for further investigations, which may have contributed to underdiagnosis. Despite ease and feasibility, operational and logistic issues should be considered for implementation of sputum induction in PHCs.[22] These include lack of skill administering the procedure, lack of facilities (hypertonic saline, suction machine, mucous extractor), specimen transfer to a laboratory, and time constraints of busy health workers. In addition, a cost-effectiveness study of sputum induction as well as Xpert MTB/RIF for TB diagnosis in children is required.

In conclusion, Xpert MTB/RIF improves case finding and quality of pediatric TB diagnosis. Sputum induction is a feasible method to collect sputum in children, but more training is required to obtain good quality specimens in PHCs and hospitals alike.

Conflict of Interest

None declared.

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Case Report

Case report on Fanconi syndrome in Wilms tumor

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Fanconi syndrome is a group of clinical manifestations including aminoaciduria, proteinuria, glycosuria, hypophosphatemia, and metabolic acidosis. It may occur after exposure to certain drugs. The most common causes are antiepileptic, antiviral, antibiotic, and antineoplastic drugs.[1] The two most common causes in the antineoplastic regimen are cisplatin and ifosfamide. Ifosfamide, a derivative of cyclophosphamide, has been used to treat pediatric solid tumors.[2] Its high efficacy in numerous studies has led to its long-term administration for pediatric malignancies, including Wilms tumor. Along with other treatment modalities, ifosfamide considerably improved the survival rate (90%) of Wilms tumor while only a few cases resulted in Fanconi syndrome.[1,3,4]

Here we illustrate a case of presumed drug induced Fanconi syndrome in a Wilms tumor patient who previously achieved remission for 10 months. **[Paediatr Indones. 2020;60:223-5; doi: http://dx.doi.org/10.14238/pi60.4.2020.223-5**].

Keywords: *Wilms tumor; Fanconi syndrome; ifosfamide; nephrectomy*

The Case

A 3-year-old boy was admitted to the hospital due to sudden weakness in both legs from the prior day. He had difficulty walking and moving his body. There was no history of trauma, falls, familial disease, or diarrhea. He had completed the polio vaccination regimen. He had been diagnosed with Wilms tumor stage IV and underwent unilateral nephrectomy, 15 cycles of chemotherapy (actinomycin, vincristine, and ifosfamide) over 50 weeks, and radiation, achieving complete remission for the previous 10 months.

The patient was alert and conscious. Vitals signs were within normal limits. Physical examination was unremarkable. Neurological examination revealed a decrease in muscle strength of both legs (point 3 of manual muscle testing). Sensory and autonomic function showed no abnormalities. Physiological reflexes were diminished and no pathological reflexes were observed in both legs. Complete blood count and biochemical parameters revealed anemia and severe

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hypokalemia, with potassium level at 1.9 mEq/L. Glomerular filtration rate was otherwise normal. Blood gas analysis confirmed the presence of metabolic acidosis. Urinalysis was done to evaluate for a possible urinary cause of these abnormalities. Glucose and protein were detected in his urine. Given his history and work up, a diagnosis of ifosfamide-induced Fanconi syndrome was suspected. He underwent potassium and bicarbonate correction. The patient was closely monitored by assessing his laboratory abnormalities. After 3 days of treatment, his general condition improved. The motor function in both legs returned to normal and the patient was discharged.

Discussion

One major concern for a survivor of Wilms tumor is nephrotoxicity. Nephrotoxicity manifests in several forms, from glomerular to tubular dysfunction or even acute renal failure.[5] It can have a primary etiology or be due to drug-induced process.

Renal tubular dysfunction is categorized into proximal and distal tubular dysfunction depending on the affected site. Proximal tubular dysfunction implies an impairment of fractional electrolyte excretions or reabsorption of substances such as amino acids, low molecular weight proteins (LMWPs), phosphate, bicarbonate, glucose, and urate. In contrast, distal tubular abnormalities are expected to impair the osmolality of the urine.[6]

Proximal tubular toxicity, known as Fanconi syndrome is a generalized reabsorption defect resulting in clinical features such as aminoaciduria, low molecular weight proteinuria, normoglycemic glycosuria, metabolic acidosis, hypokalemia, organic aciduria, hypophosphatemia, hypouricemia, and polyuria.[7] According to his urinalysis, blood work, and blood gas analysis results, our patient likely fulfilled the first five of the aforementioned conditions. In addition, glomerular damage in our patient was excluded as the glomerular filtration rate was within normal limits. Distal tubular dysfunction was not fully measured due to the lack of urine osmolality data.

Fanconi syndrome has a wide spectrum of clinical manifestations. The presentation can be growth failure, rickets, dehydration, or hypokalemia. The obvious symptom in our patient was severe hypokalemia. The low level of potassium may have been due to an increase in urinary potassium secretion, worsening with acidosis that contributes to an increase of filtered potassium load. Metabolic acidosis in the patient primarily reflects a defect in bicarbonate reabsorption in the proximal tubule.[8]

The pathomechanism by which ifosfamide, in this case, may induce Fanconi syndrome remains unclear. Ifosfamide, one of the alkylating antineoplastic prodrugs, requires CYP3A5 and CYP2B6 located in the kidney and liver, respectively, to form acrolein, chloracetaldehyde (CAA), and isophosphoramide (active drug). The CAA, the metabolite of ifosfamide, is known to induce proximal tubular cell injury. An in vitro study showed that the accumulation of CAA reduces glutathione and ATP level through inhibition of Na/K/ATPase as well as V-ATPase.[3] The ATP is essential for endocytosis and transport across the basolateral membrane.[9] A decrease in endocytosis and membrane transport automatically diminishes any reabsorption. Another study found that ifosfamide itself readily binds to the organic cation transporter (OCT) receptor and leads to a higher intake of ifosfamide in the tubular epithelial cells.[1,10]

Renal dysfunction in Wilms tumor patients may be associated with the previous treatment. Past studies revealed a tendency to acute renal failure in patients following nephrectomy. A study showed that uninephrectomised children with Wilms tumor have a higher risk of renal impairment.[11] Nephrectomy was comparable to other nephrotoxic chemotherapy (cisplatin) in inducing renal damage in patients receiving ifosfamide.[12] However, another study reported that patients treated with only unilateral radical nephrectomy were at low risk for long-term renal dysfunction.[13] The filtration rate has been postulated to increase after a reduction of renal mass, leading to the high uptake of ifosfamide in the nephrons and tubules.[4] This theory may explain the main role of the nephrotoxic regimen in diminishing renal function in our patient.

Our patient successfully achieved remission after unilateral nephrectomy combined with chemotherapy. However, durable remission suggests that ifosfamide therapy at fairly high doses (>50 g/m^2) retained the potency to cause late complications.[1] Therefore, identifying high-risk pediatric patients for acute kidney injury,[14] e.g., those with younger age, concomitant use

of other nephrotoxic agents, or history of renal failure before starting the treatment, may be mandatory in the future. In spite of the therapeutic effectiveness of ifosfamide, regular monitoring of complete renal function and electrolytes should be performed in conjunction with assessment of disease progression and further complications of Fanconi syndrome, e.g., rickets and osteomalacia.

Conflict of Interest

The authors declare no conflict of interest.

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Original Article

Brain natriuretic peptide and atrial septal defect size in children

Siti Aizah Lawang, Haryanty Kartini Huntoyungo, Dasril Daud

Abstract

Background Atrial septal defect (ASD) is one of the most common forms of congenital heart disease (CHD). Brain natriuretic peptide (BNP) is a heart marker released into the circulation during pressure overload, heart volume expansion, and increased stress on +the myocardial wall.
Objective To assess for a possible association between atrial septal defect size and BNP level in pediatric patients.
Methods This cross sectional study on children with ASD was done from March to December 2018 in pediatric outpatients and inpatients at Dr. Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi. Measurement of ASD defect was conducted using echocardiography and categorized as small defect (<3 mm), medium defect (3-8 mm), and large defect (>8 mm). Brain natriuretic peptide was measured using radioimmunoassay and immunoradiometricassay. Nutritional status was categorized using WHO if the patients aged younger than 5 years and NCHS for patients aged equal or more than 5-year-old.
Results Mean BNP levels were 65.5 pg/mL in the small ASD group, 273.2 pg/mL in the moderate ASD group, and in 654.5 pg/mL in the large ASD group, with significant differences among ASD groups. We found a significant positive correlation between BNP levels and ASD diameter (r=0.829; P=0.001), with Y regression equation of: (BNP level) =2.624 + 0.009X (ASD diameter in mm).
Conclusion Brain natriuretic peptide levels have significant positive correlation with ASD size. Hence, BNP measurements can be used to predict septal defect size in children with ASD. Acyanotic CHD patients with suspected ASD and high BNP levels may have moderate-to-large ASDs. **[Paediatr Indones. 2020;60:277-82; DOI: 10.14238/pi60.5.2020.277-82].**

Keywords: *atrial septal defect size; brain natriuretic peptide*

Congenital heart disease (CHD) is the most common heart disorder affecting infants and children. It is found in 8 of every 1,000 live births, with one-third manifesting as a critical condition in the first year of life, and causing death in 50% of emergencies in the first month of life.[1] A Taiwanese study showed a slightly higher prevalence of 13.08 of 1,000 live births, in which around 12.05 occurred in male babies. The most common CHD is ventricular septal defect.[2] Congenital heart disease is divided into two types, cyanotic and acyanotic. Cyanotic CHD is characterized by central cyanosis due to right-to-left shunts, including tetralogy of Fallot (TF), transposition of the great arteries (TGA), and double-outlet right ventricle (DORV). Acyanotic CHD can be divided into three groups based on hemodynamics: (1) left-to-right shunts such as persistent ductus arteriosus (PDA), atrial septal defect (ASD), and ventricular septal defect (VSD); (2) right

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heart obstruction such as pulmonary valve stenosis; and (3) left heart obstruction such as aortic valve stenosis, coarctation of the aorta, and mitral stenosis.[3]

Atrial septal defect is the second most common CHD, with an incidence of around 18% of all CHDs. Hemodynamic disorders that occur in ASD are caused by left-to-right shunts due to a defect in the atrial wall of the heart. As a result, blood from the left atrium, which is supposed to enter the left ventricle, enters the right atrium then the right ventricle. If the hole is large enough, it can increase the load volume of the right heart, while also increasing the load volume of the left heart. There are three types of ASD: secundum ASD (50-70%), primum ASD (30%), and sinus venous type ASD (10%).[3]

B-type natriuretic peptide (BNP) is a cardiac neurohormone originating from the granular membrane of the heart ventricles, which contributes to increased ventricular volume and high pressure. The BNP was initially found in pig brain and named brain natriuretic peptide (BNP). While this substance is also found in human brain, it is more commonly found in the heart, secreted by ventricular cardiomyocytes in response to increased tension of the heart muscle wall and pressure on ventricular filling. Therefore, BNP levels may have a correlation with defect size in ASD. The BNP examination has been suggested as a method for early detection of ASD complication. Echocardiography is typically used to diagnose CHD, but it is not readily available and is operator-dependent. Therefore, in order to detect and manage ASD complications in a timely manner, we aimed to assess for a relationship between BNP levels and heart defect size, with the hope of using BNP as a marker for ASD. With early treatment, children's morbidity and mortality can potentially be reduced.[4] We aimed to assess for a possible association between atrial septal defect size and BNP level in pediatric patients.

Methods

This cross-sectional study was conducted at Dr. Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, from March to December 2018. The independent variables were acyanotic CHD with large ASD < 3 mm, medium ASD 3-8 mm, and small ASD > 8 mm. The BNP examination was conducted at *Hasanuddin University Medical Research Centre* (HUMRC) using immunoradiometricassay (IRMA) method. Venous blood sample was collected without fasting before and 5-10 mL blood was stored into the tube without anticoagulation. The blood then was centrifuged with 3000 rpm speed for 10 minute, afterward the serum stored in the freezer (-200C).

Nutritional status for subjects aged > 5-years-old was classified using *NCHS*: well-nourished (actual weight/ideal height according to age 90-110%, undernourished (actual weight/ideal height according to age 70-< 90%, malnourished (actual weight/ideal height according to age < 70%). While for subjects aged ≤ 5-years-old was classified using *WHO*: well-nourished (weight for height -2 SD to +2 SD according Z score; undernourished weight for height -2 SD to -3 SD according Z score; malnourished (weight for height < 3 SD according Z score).

The subjects were recruited from pediatric outpatients and inpatients with ASD at Dr. Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, who met the inclusion criteria. Inclusion criteria were children with ASD 1 month-18 years of age whose agreed to join this study. Exclusion criteria were ASD with renal disease, sepsis, cancer, pulmonal stenosis, total anomalous pulmonary venous drainage (TAPVD). Subjects' data on age, sex, nutritional status, echocardiography, and BNP levels were collected from medical records. Echocardiographic results were interpreted by pediatric cardiologists, then validation and reliability were tested in the interpretation of electrocardiogram results.

Data were grouped based on size of defect small, moderate and large. The appropriate statistical methods were then chosen: univariate (mean, median, frequency and standard deviation) and multivariate analysis (one way anova test, Pearson correlation test). This study was approved by the Ethics Committee of the Universitas Hasanuddin Medical School.

Results

During the study period, there were 103 subjects consisted of 58 females (56.3%) and 45 males (43.7%). The mean age of subjects was 3 years 6 months, ranging from 6 months to 13 years 3 months. Subjects'

nutritional status was classified as malnourished (50; 48.5%), undernourished (27; 26.2%), or well-nourished (26; 25.2%) (**Table 1**).

Table 1. Subjects' characteristics

Characteristics	(N= 103)
Age, months	
Mean (SD)	43.74 (37.67)
Median (range)	28.00 (6-159.00)
Gender, n (%)	
Female	58 (56.3)
Male	45 (43.7)
Nutritional status, n (%)	
Well-nourished	26 (25.2)
Undernourished	27 (26.2)
Malnourished	50 (48.5)
ASD defect size, n (%)	
Small	33 (32)
Medium	35 (34)
Large	35 (34)
ASD diameter, cm	
Mean (SD)	5.5 (3.02)
Median (range)	5.7 (1.2-12.70)
BNP level, pg/mL	
Mean (SD)	336.24 (294.33)
Median (range)	214 (50-979.00)

Statistical analysis showed that there were significant differences between the mean BNP levels in the malnutrition group for malnutrition and good nutrition (**Table 2**).Bonferroni's post-hoc analysis revealed significant differences between mean BNP level differences in the undernourished *vs.* malnourished groups (397.756 pg/mL; P<0.000) and the malnourished *vs.* well-nourished groups (471.229 pg/mL; P<0.000). The mean BNP level difference between the undernourished *vs.* well-nourished groups was 73.472 pg/mL (P<0.00) (**Table 3**).

Table 2. BNP level based on nutritional status

BNP level, pg/mL	Nutritional Status		
	Malnourished (n = 50)	Under-nourished (n = 27)	Well-nourished (n = 26)
Mean	559.5	161.7	88.2
Median	609.0	100	63.0
SD	252.11	151.8	91.6
Range	67-979	50-577	50-454

One-way Anova test=62.607; P=0.001

Statistical analyses revealed significant differences in mean BNP level differences among the small, medium, and large ASD groups [small *vs.* medium (207.654 pg/mL; P<0.00), small *vs.* large (588,968 pg/mL; P<0.00), and moderate *vs.* large (381.314 pg/mL; P<0.00)] (**Table 4**).

Multivariate regression analysis was done on the two variables with significant correlations to BNP levels by univariate analyses. We found that ASD size and BNP level had a significant association (P=0.001), but nutritional status and BNP level did not (P=0.086) (**Table 5**).

Y regression equation of BNP level was 2.624 + 0.009X (ASD diameter in mm). Correlation analysis between BNP levels and ASD diameter showed that the correlation between BNP levels and ASD diameter was significant with P=0.000 (P<0.05) and r=0.829 with the Y regression equation (BNP level) =2.624 + 0.009X (ASD diameter in mm) (**Figure 1**). This finding indicates that using BNP level to estimate ASD size is equivalent to echocardiographic ability, with a regression equation of a 1 mm increase in ASD diameter for a 0.0009 pg/mL BNP increase. Spearman's correlation analysis of BNP level and ASD diameter revealed a significant correlation (P=0.0001and r=0.829) (**Figure 1**).

Discussion

Atrial septal defect is a common type of congenital heart disease (CHD) in children, with a prevalence of approximately 10-15% of CHD patients. Atrial septal defects can occur alone or in combination with other cardiac defects. Most children with ASDs do not show any clinical symptoms during childhood. However, some common clinical signs in pediatric patients with ASD are systolic ejection murmur and wide-fixed split. Similar heart sounds are often heard in children without ASD or other CHD types, which makes diagnosing ASD a challenge for clinicians. Thus, echocardiography is crucial in determining a diagnosis, in addition to history-taking and physical examination.[5]

In our study, ASD was more common in girls than boys (56.3% *vs.* 43.7%, respectively). A study in Taiwan also reported more CHD (ASD) in boys than girls, and there was no significant relationship

Table 3. Comparison of mean BNP level differences by nutritional status

Nutritional status	Difference in mean BNP level, pg/mL	95% CI	P value
Malnourished *vs.* undernourished	397.76	282.57 to 512.95	0.00
Malnourished *vs.* well-nourished	471.23	354.61 to 587.85	0.00
Undernourished *vs.* well-nourished	73.47	-59.05 to 206.56	0.54

Table 4. Comparison of mean BNP level differences and ASD size

ASD size	Difference in mean BNP level, pg/mL	95% CI	P value
Small *vs.* medium	207.654	109.961 to 305.347	0.000
Small *vs.* large	588.968	491.275 to 686.661	0.000
Medium *vs.* large	381.314	285.068 to 477.560	0.000

Table 5. Multivariate logistic regression analysis on BNP level with nutritional status and ASD size

Variables	B	SE	P value	95% CI
Nutritional status	-56.380	32.552	0.086	-120.961 to 166.595
ASD diameter	250.112	33.132	0.001	184.378 to 315.846
Constant	-69.215	118.858	0.562	-305.026 to 166.595

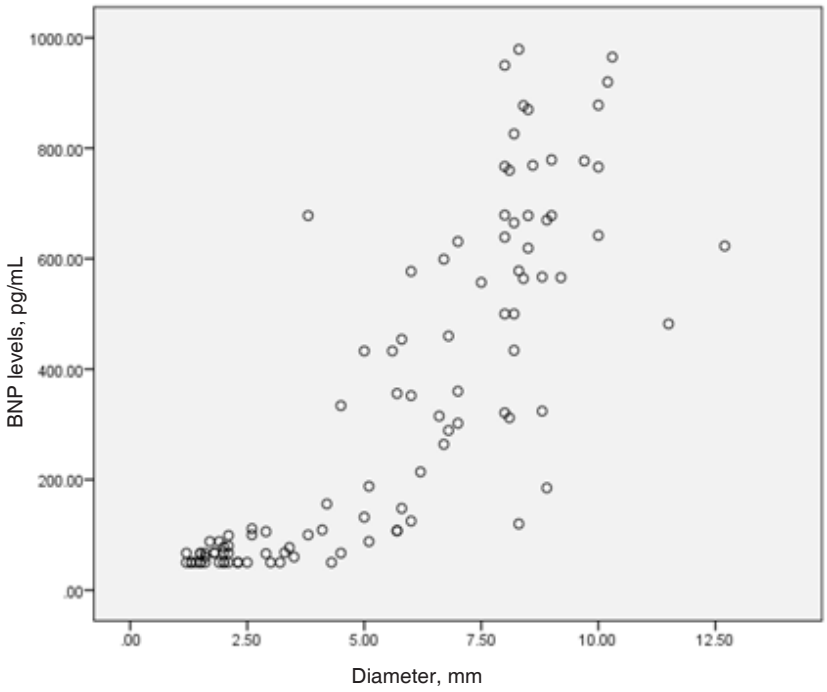

Figure 1. Correlation of BNP levels and ASD diameter

between sex and the size of the defect (P=0.605).[2] Our subjects' mean age was 3 years 6 months, with a median of 1 year 4 months. Mean ages of children according to defect size were 31.12 months for small, 32.4 months for moderate, and 67 months for large. One-way ANOVA test showed significant differences in age and ASD Size between small, medium, and large ASD (P=0.001).

In our study, nutritional status was classified as well-nourished (25.2%), undernourished (26.2%), and malnourished (48.5%). Similarly, a study in Jakarta showed a malnutrition prevalence in children with CHD of 51.1%, with 22.3% of them are malnourished.[6] In our study, there was a significant relationship between the size of the defect and the nutritional status of the patients (P=0.001). Poor nutritional status was more common in large ASD patients, while good nutritional status was more common in small ASD patients.[6] Our ASD subjects were categorized as having small ASD (33 subjects; 32%), moderate ASD (35 subjects; 34%) or large ASD (35 subjects; 34%). A previous study reported small ASD in 39 subjects (50%), moderate ASD in 15 (19.23%), and large ASD in 24 (30.77%).[7]

B-type natriuretic peptide is a cardiac neurohormone originating from the granular membrane of the heart ventricles, which contributes to the increase in ventricular volume and blood pressure. On statistical tests, there was significant difference in the average difference in BNP levels in malnourished and poor nourished as well as malnourished and good nourished with the value of P=0.001. Similarly, a previous study reported a correlation in malnourishment and higher BNP levels in children.[8] We found significantly higher mean BNP in the medium ASD group compared to the small ASD group, the large ASD group compared to the small ASD group, as well as the large ASD group compared to the medium ASD group. A study also noted a correlation between BNP level and ASD size.[9] In addition, a study showed higher BNP levels in their ASD group (79 pg/mL) compared to a control group (57 pg/mL) (P<0.05).[10] This shows that BNP levels can match echocardiography in diagnosing the size of ASD.

Bivariate analysis revealed that nutritional status and ASD diameter had significant correlations with BNP level. However, multivariate analysis revealed that only ASD diameter was significantly correlated with BNP level. This finding indicates that using BNP level to estimate ASD size is equivalent to echocardiographic ability, with a regression equation of a 1 mm increase in ASD diameter for a 0.0009 pg/mL BNP increase. Another study stated that serum BNP levels were higher in ASD patients compared to the control group (healthy subject), all of whom had been examined using echocardiography.[7] A previous study likewise found that serum BNP levels were higher in patients with larger ASDs.[8] These studies suggest that BNP levels can be used as a diagnostic tool to predict ASD size.

Some subjects in our study had low BNP levels accompanied by moderate or large ASDs. These low BNP levels may have been due to the absence of adaptation or compensation for the enlargement of the cardiac chamber; the resulting defects also have not shown any significant hemodynamic disorders. In addition, the enlargement of heart space is also influenced by age and nutritional status.[6]

The strength of our study was we determine the cut off point the size of ASD and relation with the BNP levels. Also, echocardiographic results were interpreted by pediatric cardiologists, then validation and reliability were tested in the interpretation of electrocardiogram results. In addition, this study was conducted at Dr. Wahidin Sudirohusodo Hospital, which is a national referral center in Eastern Indonesia, so our results are representative of ASD children in Eastern Indonesia. The limitation of this study was that BNP measurements were only done once.

The application of our study results is that in patients with clinically acyanotic CHD who are suspected of ASD, high BNP level is a strong indicator of moderate-large ASD. Such patients require immediate treatment, so early diagnosis is needed. In addition, BNP levels could also be used to monitor the success of further treatment. Patients with systolic ejection noise, wide-fixed split, and low BNP level are likely to have a small ASD, but such conditions do not rule out the presence of a moderate ASD, so these patients must be urgently referred for echocardiography. Therefore, history-taking, physical examination, and BNP measurements are needed by primary care physicians to be able to diagnose CHD early and determine when patients should be referred immediately to tertiary services.

Conflict of Interest

None declared.

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Original Article

Cost analysis of congenital heart disease patients who underwent diagnostic catheterization

Ni Putu Wirantari, Eka Gunawijaya, Ni Putu Veny Kartika Yantie

Abstract

Background Cardiac catheterization has developed into an important technique for diagnosis and management of congenital heart disease (CHD) patients. Catheterization is expensive and almost all patients who undergo the procedure at Sanglah General Hospital are participants of the *Social Insurance Administration Organization* (*Badan Penyelenggara Jaminan Sosial/BPJS*) that uses the *Indonesian Case-Based Groups* (INA-CBGs) payment system.
Objective To determine the characteristics and analyze costs of CHD patients who underwent diagnostic catheterization.
Methods This retrospective study used patient medical record data from March 2009 - July 2018 in Sanglah Hospital, Bali. Data collected included CHD type, age, sex, weight, height, nutritional status, length of procedure, complications, hospital rates, and INA-CBG rates. Data analysis was done with *SPSS software*.
Results Of 219 CHD patients who underwent non-intervention catheterization, most had cyanotic CHD. Catheterization intervention in 2018 showed a discrepancy between the INA-CBG rate and hospital rate. The biggest difference was 107%, in patients who underwent mild heart intervention with class 3 of treatment.
Conclusion Most subjects are diagnosed with cyanotic CHD especially tetralogy of Fallot and most has already received intervention. There are negative differences between the INA-CBG rates and the hospital real rates for catheterization. **[Paediatr Indones. 2020;60:244-52 ; DOI: 10.14238/pi60.5.2020.244-52]**.

Keywords: *heart catheterization; congenital heart disease; INA-CBG; cost analysis; National Health Insurance*

Congenital heart disease (CHD) is a heart disorder that has a significant impact on the morbidity, mortality, and health costs of children. Children with CHD have structural abnormalities in the heart and/or large blood vessels that appear at birth.[1,2] The incidence of CHD is estimated to be 6-8 per 1,000 live births in the general population. CHD events were reported to be 4-10 per 1,000 births in the United States, 6.9 per 1,000 births in Europe, and 9.3 per 1000 births in Asia.[3,4] A study noted that out of 903 catheterizations performed, 51% were diagnostic, 43% were interventions, and 6% were for endomyocardial biopsies.[5]

Cardiac catheterization is an important procedure for diagnosis and management of CHD patients. The role of cardiac catheterization required as a diagnostic tool because of its accuracy and ability to directly assess the cardiac space.[6] Diagnostic catheterization in acyanotic CHD serves to describe the complete anatomy of the heart and assess pulmonary arterial pressure. Catheterization in cyanotic CHD is

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used to assess pulmonary artery anatomy, pulmonary pressure, and pulmonary aortic collateral vessels.7

The *National Health Insurance* (*Jaminan Kesehatan Nasional/JKN*) was first introduced by the Indonesian government on January 2014. This insurance guarantees health protection by the government for participants who have paid their fees. This program is organized by the *Social Insurance Administration Organization* (*Badan Penyelenggara Jaminan Sosial/BPJS*), which was a change from previous health insurance for government employees namely *Health Insurance (Asuransi Kesehatan/ASKES)*.[8] The Presidential Regulation no. 111 (2013) was a revision of Presidential Regulation no. 12 (2013) concerning health insurance, with regards to payments for health services by BPJS using the *Indonesian Case-Based Groups* (INA-CBG) coding and reimbursement system in 2014.[9]

The INA-CBG is a system for determining standard rates used by the hospital as a reference to claim BPJS reimbursements to the government. The INA-CBG rate is the reimbursement rate from BPJS paid to advanced health facilities for service packages based on grouped diagnoses of disease and procedures. The rate is in the form of a package including all components of the hospital rate. Rate data and disease coding refer to the *International Classification of Diseases* (ICD) regulated by the *World Health Organization* (WHO). The ICD 10 includes 14,500 diagnostic codes and the clinical ICD 9 includes 7,500 codes. The INA-CBG rates are subdivided according to 6 types of hospital classes, namely D class hospital, C class hospital, B class hospital, A class hospital, public hospitals, and national referral hospitals. The INA-CBG rate is arranged based on treatment classes, namely, classes 1, 2, and 3.[10,11]

To date, there have been no studies on the cost of catheterization in CHD patients at Sanglah General Hospital, Denpasar. Hence, we aimed to assess the characteristics of such patients and compare the hospital procedure costs to the reimbursement rate from BPJS.

Methods

This retrospective study included CHD patients who underwent diagnostic catheterization at Sanglah General Hospital, Bali, from March 2009 - July 2018. We collected data from patient medical records. Patients with incomplete medical records were excluded. The study was approved by the Research Ethics Commission of the Medical School Udayana University/Sanglah General Hospital, Denpasar.

Data from the study included CHD type, age, sex, weight, height, nutritional status, length of procedure, complications, hospital rates, and INA-CBG rates. Operational definitions of variables were as follows:

1. Congenital heart disease (CHD) was a heart disorder present at birth. This CHD was divided into two types, namely, cyanotic and acyanotic heart disease. Cyanotic CHD was characterized by central cyanosis due to the presence of right-to-left shunts, for example, tetralogy of Fallot, transposition of large arteries, or tricuspid atresia. Acyanotic CHD was typified by leakage of the heart septum accompanied by left-to-right shunts, including ventricular septal defect (VSD), atrial septal defect (ASD), or blood vessel openings as in the persistent ductus arteriosus (PDA). In addition, acyanotic CHD was also found in obstruction of the ventricular outlet such as aortic stenosis, pulmonary stenosis, and coarctation of the aorta. Acyanotic CHD was divided into isolated and non-isolated. Isolated was CHD with single abnormality and non-isolated with combination abnormalities.[12]
2. Diagnostic cardiac catheterization was the act of inserting a small tube (catheter) into the arteries and/or veins and tracing it to the heart, other blood vessels and/or other organs that were targeted with the aid of X-rays aimed at diagnostics (seeking interference structure and/or function of the heart blood vessels, other blood vessels, and/or other organs).[13]
3. Age was based on the date of birth taken from subjects' medical records, expressed in years. Age calculation using benchmark of 12 months for one year. If the age calculation was less than 6 months of age, it was rounded down to zero and if more than or equal to 6 months, then it was rounded up to the nearest whole number, and expressed on a numerical scale.
4. Weight was measured using a scale in units of kilograms (kg). Children aged 1 to 24 months were weighed using infant scales, whereas children

over 24 months were weighed with a pediatric standing balance. Body weight was recorded with accuracy to 0.05 kg in infants and 0.5 kg in older children, expressed in kg.

5. Height was measured by scales in centimeters (cm). Measuring the body length of children aged 6 to 12 months was done by two people to ensure that the baby's head touched the head restraint board in the Frankfort (Frankfort horizontal line) flat plane. For children over 12 months, height was measured in a standing position, using a stadiometer. When measuring height, the child stood upright with thighs touching side-by-side, barefooted, and heels, buttocks, and back of the head touching the stadiometer. Height was expressed in cm.
6. Nutritional status was assessed by comparing actual body weight to ideal body weight (based on the 2007 WHO Curve. Patients were classified as obese for >120%, overweight for 110 to 120%, well-nourished for 90 to <110%, under-nourished for 70 to <90%, severe malnutrition for <70% according to Waterlow,[14] expressed as a categorical scale.
7. The length of the procedure was the length of time starting from injection of the arteries until the catheter was removed.
8. Complications of catheterization consisted of major and minor complications. Major complications were death, life-threatening hemodynamic decompensation (cardiac arrest or severe hypotension), those that required surgical intervention (tool embolization), or resulted in anatomical lesions, or permanent function events (cerebral infarction, permanent arterial thrombosis, damage to blood vessels, aneurysms). Minor complications consisted of transient events that resolved with or without therapy (e.g., treatable arterial thrombosis and transient arrhythmias).[15,16]
9. Hospital rate was the total cost of hospital care including medicines, medical equipment, paramedics, doctors, wards, laboratory and radiology examinations, and administration fees. The INA-CBG rate was the payment amount by BPJS to advanced health facilities for the service package that was claimed, based on the classification of disease diagnosis and procedures.[8,9] Discrepancy defined as margin between hospital rate with INA CBG rate.
10. The severity of cases in INA-CBG was divided into mild, moderate, and severe. The mild category was used for cases of hospitalization with severity 1 (without complications or comorbidities), moderate for cases of hospitalization with severity 2 (with complications and mild comorbidities), and severe for cases of hospitalization with severity 3 (with complications and severe comorbidities).[9]
11. The INA-CBG rate was arranged based on treatment classes, namely, classes 1, 2, and 3. [10,11]

Data analysis was done with *SPSS software*. Descriptive data were depicted in the form of images, tables, and narratives. Ethical approval was obtained from the Health Research Committee, Sanglah Hospital/Universitas Udayana Medical School.

Results

Of 219 catheterization subjects, 100 subjects had surgery. Of 119 subjects who did not undergo surgery, 2 were treated conservatively, 21 were referred to Harapan Kita Hospital, Jakarta, 6 negotiated about further treatment, 4 refused further treatment, 13 died, and 73 were lost to follow up (**Figure 1**).

The number of subjects who underwent catheterization at Sanglah General Hospital from March 2009 - July 2018 was 219 (**Figure 2**). As shown in **Table 1**, the 0-5 year age group had the most subjects. Males predominated with 53.9%. The most common type of heart disease was cyanotic CHD (51.1%), with several diagnoses including double outlet right ventricle (DORV) (accompanied by atrial septal defect/ASD, tricuspid atresia/TA, transposition of the great arteries/TGA, pulmonary stenosis/PS), pulmonary atresia/PA (accompanied by ASD, TA, ventricular septal defect/VSD), TGA (accompanied by ASD, atrioventricular septal defect/CAVSD, PA, VSD), or tetralogy of Fallot/TF (accompanied by PDA, PA, ASD). Median radiation time was 13.3 minutes in acyanotic patients and 14.3 minutes in cyanotic patients.

Figure 3 shows that ventricular septal defect is the most case (63 cases) from isolated acyanotic CHD category. From non-isolated acyanotic CHD

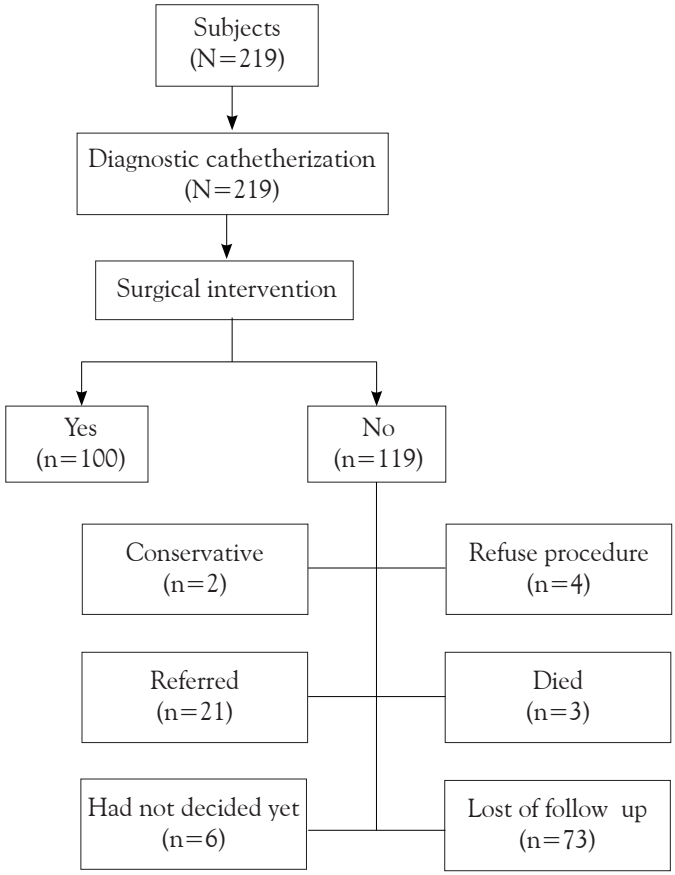

Figure 1. Flow chart of catheterization patients in March 2009 - July 2018

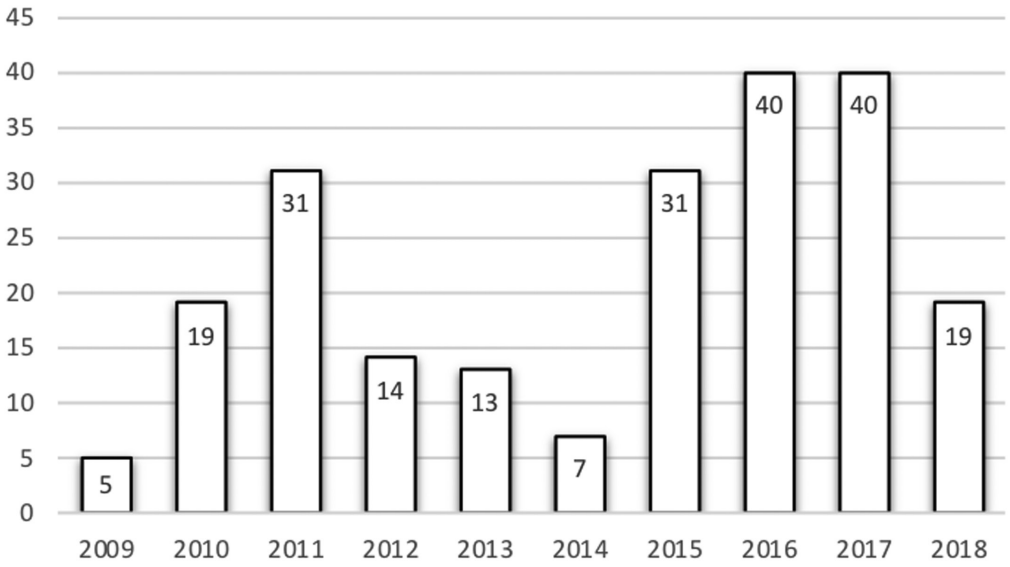

Figure 2. Diagnostic catheterizations at Sanglah Hospital

Table 1. Subjects' characteristics

Characteristics	(N=219)
Age, n (%)	
0-5 years	149 (68)
6-10 years	43 (19.6)
11-18 years	27 (12.3)
Gender, n (%)	
Male	118 (53.9)
Female	101 (46.1)
Median body weight (range), kg	11.0 (2-55)
Mean height (SD), cm	94.9 (27.2)
Nutritional status, n (%)	
Obese	9 (4.1)
Overweight	13 (5.9)
Well-nourished	75 (34.2)
Under-nourished	70 (32.0)
Severe malnutrition	52 (23.7)
Type of CHD, n (%)	
Cyanotic	112 (51.1)
Acyanotic	107 (48.9)
Isolated	90
Non-isolated	17
Type of anesthesia, n (%)	
General	219 (100)
Local	0 (0)
Complications, n (%)	
Major	9 (4.1)
Minor	102 (46.6)
None	108 (49.3)
Median duration of radiation (range), minutes	13.9 (1.8-71.0)
Median duration of procedure (range), minutes	69.0 (15.0-213.0)

category, VSD + PDA are the most cases on this study (8 cases) as showed on **Figure 4**. **Figure 5** shows that ToF + varian are the most cases (61 cases) on cyanotic CHD category.

Table 2 shows the difference between hospital rate and INA CBGs rate for cases in year 2018. The biggest difference was found in mild case with class 3 inpatient room (107%). Among 219 subjects, we randomly choosed 13 subjects from year 2018 database as examples for discrepancy (**Table 2** & **Table 3**). We choosed the year of 2018 as the BPJS database was started on this year. We could not calculated thehospital losses for 9 years (between 2009-2018) since the data was not available.

Discussion

In our study, most subjects were aged 0-5 years. It is slightly different with a study in Sarajevo who observed that the average age of pediatric patients with CHD who underwent cardiac catheterization was 5.9 years. They also noted that more male CHD patients underwent diagnostic catheterization (58.3%).[17] Our subjects were also comprised of more males (118; 53.9%) than females (101; 46.1%).

Our cases had cyanotic CHD (51.1%) with TF as the major diagnosis. A previous study reported that 67% of their subjects had cyanotic CHD during

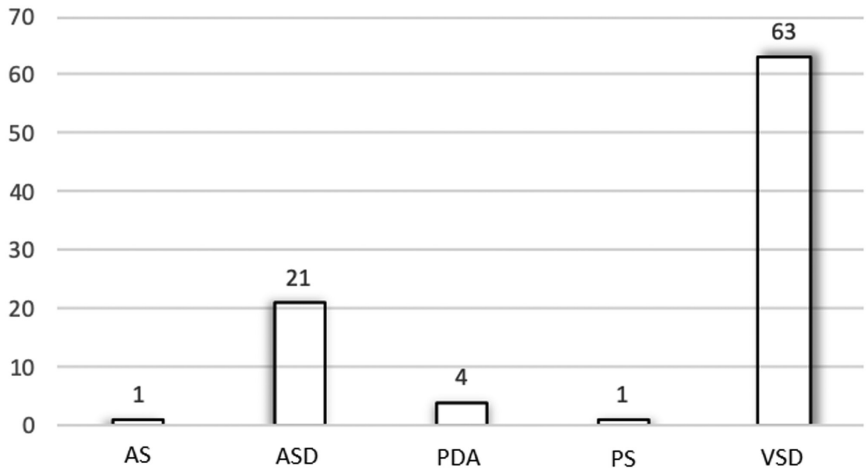

Figure 3. Isolated acyanotic CHD
AS=aortic stenosis, ASD=atrium septal defect, PDA=patent ductus arteriosus, PS=pulmonal stenosis, VSD=ventricle septal defect.

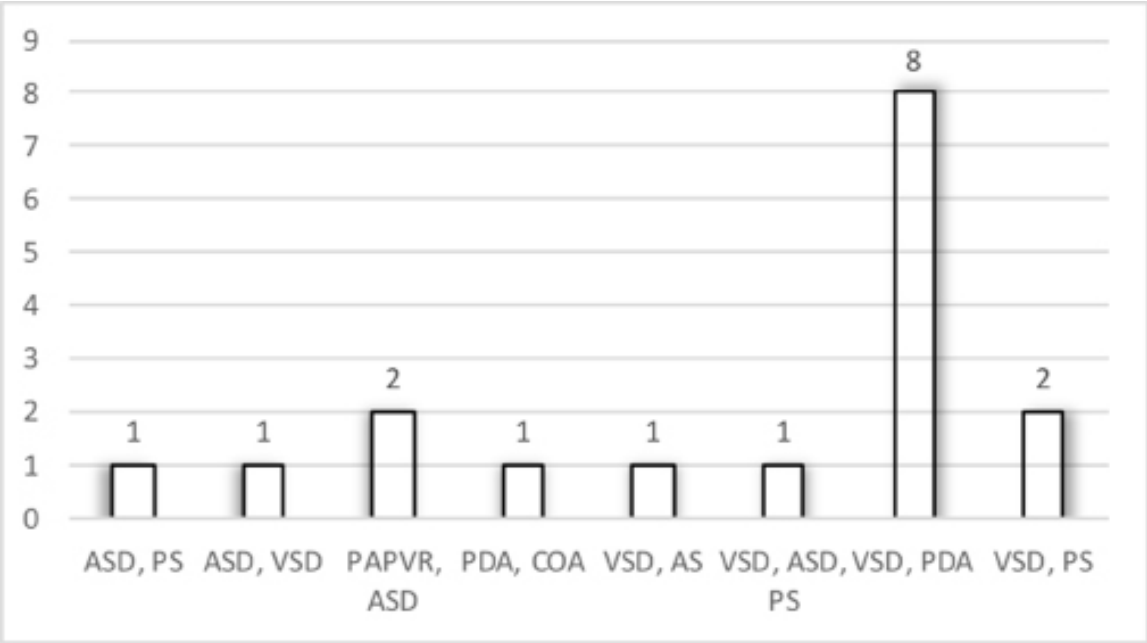

Figure 4. Non-isolated acyanotic CHD
PAPVR=partial anomalous pulmonary venous return, COA=coarctation of the aorta

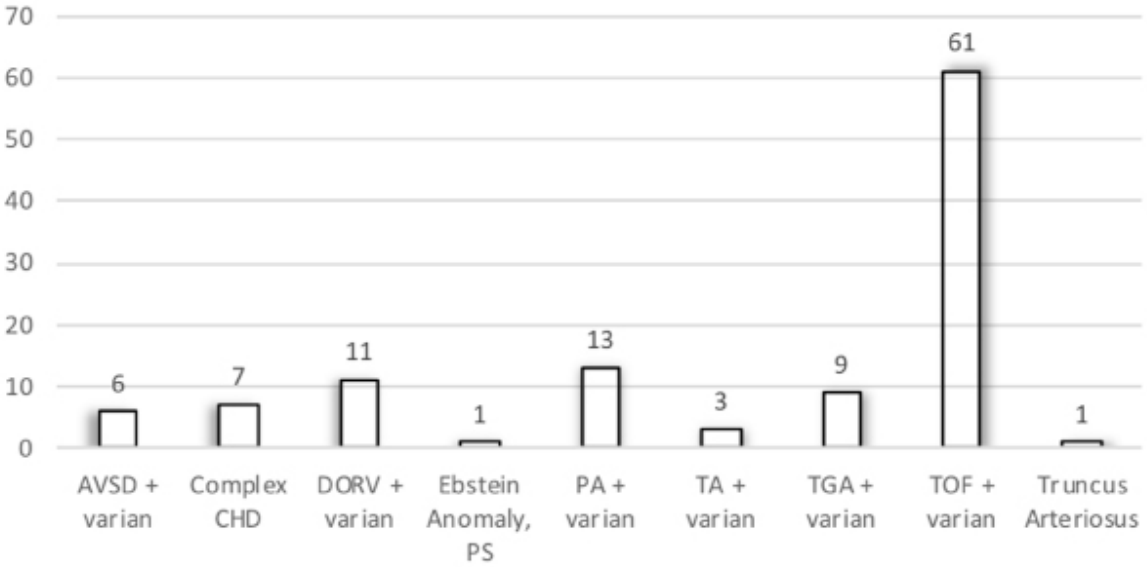

Figure 5. Cyanotic CHD
AVSD=atrioventricular septal defect, complex CHD=complex congenital heart disease, DORV=double outlet right ventricle, PA=pulmonary atresia, TA=tricuspid atresia, TGA=transposition of great arteries, ToF=tetralogy of Fallot

Table 2. Differences in hospital and INA-CBG rates in 2018

Catheterization	Hospital rate	INA-CBG rate	Rate difference	Percentage rate difference
Mild				
Class 3 (n=5)	12,746,163 IDR	6,165,900 IDR	6,580,263 IDR	107%
Class 2 (n=5)	14,976,036 IDR	7,399,000 IDR	7,577,037 IDR	102%
Class 1 (n=2)	14,278,518 IDR	8,632,200 IDR	5,133,184 IDR	59%
Moderate				
Class 3 (n=1)	13,743,684 IDR	8,610,500 IDR	5,133,184 IDR	59%

Table 3. Differences in hospital and INA-CBG rates based on diagnoses in 2018

Diagnosis	Catheterization procedure	Hospital rate	INA-CBG rate	Rate difference	Percentage rate difference
Isolated ASD (n=3)	Mild				
	Class 3	14,163,545 IDR	6,165,900 IDR	7,997,645 IDR	129%
	Class 2	18,608,510 IDR	7,399,000 IDR	11,209,510 IDR	151%
Isolated VSD (n=3)	Mild				
	Class 2	14,060,274 IDR	7,399,000 IDR	6,661,274 IDR	90%
	Class 1	15,008,521 IDR	8,632,200 IDR	6,376,321 IDR	74%
TF (n=2)	Mild				
	Class 3	13,680,993 IDR	6,165,900 IDR	7,515,093 IDR	122%
	Class 2	13,636,886 IDR	7,399,000 IDR	6,237,886 IDR	84%
Isolated AVSD (n=1)	Mild				
	Class 2	14,514,241 IDR	7,399,000 IDR	7,115,241 IDR	96%
DORV + variant (n=1)	Mild				
	Class 3	14,479,043 IDR	6,165,900 IDR	8,313,143 IDR	135%
PA + variant (n=2)	Mild				
	Class 1	13,548,514 IDR	8,632,200 IDR	4,916,314 IDR	57%
	Moderate				
	Class 3	13,743,684 IDR	8,610,500 IDR	5,133,184 IDR	60%
TGA + variant (n=1)	Mild				
	Class 3	7,243,690 IDR	6,165,900 IDR	1,077,790 IDR	175%

diagnostic catheterization with the majority of cyanotic CHD cases were due to TF.[7]

All pediatric CHD patients who underwent cardiac catheterization used general anesthesia. Other literature also stated that most cardiac catheterization procedures are performed under general anesthesia, to reduce or eliminate discomfort and to ensure patient cooperation during the procedure.[18]

The median radiation time in our subjects was 13.9 minutes, ranging from 1.8 to 71 minutes. Similarly, a study showed radiation duration of 5.2-39 minutes. The longest duration of radiation during catheterization was in patients with PA, TF, and PS. Children have higher radio-sensitivity compared to adults, thus, radiation exposure in the first 10 years of life has a risk factor for long-term effects, several times higher compared with that of an adult. The catheterization procedure is carried out with all precautions to minimize the effects of radiation on the operator and patient, so that the fluoroscopy time is kept as low as possible.[19] The median radiation time was 13.3 minutes in acyanotic patients and 14.3 minutes in cyanotic patients. Kumar et al. found that radiation time ranged from 2.4 to 11 minutes in acyanotic patients and 8-28 minutes in cyanotic patients. Catheterization in cyanotic CHD has a longer radiation time because of the need to assess cardiac chamber pressure, pulmonary artery anatomy, and pulmonary aortic collateral vessels.[7]

Our subjects median duration of the diagnostic heart catheterization procedure was 69 minutes. The longest duration was 213 minutes in VSD cases with ST depression during the intervention. Similarly, a previous study reported an average length of cardiac catheterization to be 118 (SD 40.2) minutes.[20] The duration of the procedure depends on the type and complexity of the abnormality, the anesthetic procedure, the experience and skill of the operator and assisting staff.

We found major complications in 4.1% of our subjects, minor complications in 46.6%, and no complications in 49.3%. In contrast, a study noted that 17% of cases had complications during catheterization. The most common complication in this study was limited bleeding at the location of insertion of catheterization access.[20]

Diagnostic catheterization from January to July 2018 consisted of mild and moderate catheterization. Table 2 shows that INA-CBG rates were lower than hospital costs in all categories of action. A previous study also showed that insurance rates were lower than hospital rates for certain diseases.[21] The BPJS officials reported that at the start of the INA-CBG package implementation, around 94 hospitals had a surplus from using INA-CBG reimbursements compared to previously; this applied to hospitals in and outside Jakarta, such as *Rumah Sakit Islam* in Jemursari, Surabaya, East Java.[9] Low INA-CBG reimbursement rates compared to hospital rate result in hospital losses, whereas INA-CBG rate higher than hospital rates benefit the hospital.

The biggest difference in rates was found in mild intervention with class 3 of treatment (107%). Factors that cause differences between hospital and INA-CBG package rates include administrative fees, accommodation, doctor's actions, nursing, pharmacy, and laboratory examinations. Hospital rate are calculated from details of the type of service, with rates determined by local regulations. Hospital rate calculations are generally based on a retrospective rate calculation, which means the rates are billed after the service is done. Such calculations do not encourage efficiency. The INA-CBG rate is determined based on prospective calculations, so it is important to establish standard disease management procedures with clinical pathways. These procedures can help the hospital team to perform optimal, efficient, and effective services in the JKN era.[11]

The INA-CBG rates are calculated based on the diagnostic code and procedure code entered into the CBG's standard code set by the central government. While hospital rates depend on patient length of stay, the INA-CBG rate is not affected because its reimbursement rate is adjusted according to only the diagnostic and procedural codes. In our study, the average length of stay was 2.1 days. The accuracy of the diagnosis and coding procedures affects the accuracy of INA-CBG rates. Coding accuracy is compared to diagnoses stated in the patient summary and audited by the BPJS verifier.[11]

Based on the results of this study, we expect that hospitals will be more selective in carrying out future catheterization measures. Other modalities that are less expensive and easier in the diagnosis of CHD should be considered as alternatives to catheterization. Other tools that can be used repeatedly and reduction of consumable items during catheterization would be preferred.

The limitations of this study were not analyzing the hospital and INA-CBG rate components. This study relatively had small number of subjects and more studies with a large number of subjects are needed to be more representative. We also did not assess the types of laboratory examinations or imaging, information on the suitability of diagnoses or coder understanding in coding appropriate diagnoses or procedures with patient records.

In conclusion, the majority of subjects have cyanotic CHD compared to acyanotic CHD, with TF as the most common diagnosis. All patients undergo catheterization using general anesthesia. Most of the catheterizations are uncomplicated. Most of the subjects have surgery at Sanglah General Hospital. There is a discrepancy between hospital rate and INA-CBG rates in diagnostic catheterization.

Conflict of Interest

None declared.

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Original Article

Validation of PELOD-2 score as a predictor of life-threatening organ dysfunction in pediatric sepsis

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Abstract

Background *The Third International Consensus Definitions for Sepsis and Septic Shock* (Sepsis-3) defined sepsis as life-threatening organ dysfunction due to immune dysregulation against infection. It recommends the *Sequential (sepsis-related) Organ Failure Assessment* (SOFA) score to evaluate life-threatening organ dysfunction. But the SOFA tool has not been adjusted for pediatric patients. The *Indonesian Pediatrics Society* (IPS) uses the same sepsis definition and recommends using the PELOD-2 score as an indicator of life-threatening organ dysfunction in children.

Objective To evaluate the validity of the PELOD-2 score for predicting life-threatening organ dysfunction in pediatric sepsis.

Methods A prospective cohort study was conducted in children with sepsis who were admitted to the PICU. Subjects were taken consecutively with inclusion criteria of 1 month-18 years of age, with organ dysfunction, having two or more symptoms of systemic inflammatory response syndrome (SIRS), and suspected or proven infection. PELOD-2 score, with and without lactate result, of each subject were plotted to receiver operating characteristic (ROC) curve, then we determined the most optimal cut off point to predict the life-threathneing organ dysfunction in pediatric sepsis based on the sensitivity and specificity of each score.

Results Sixty-six patients were analyzed, with 40 males and 26 females aged 2 to 183 months (median 11 months). Twenty patients died while in the PICU. A PELOD-2 score (with lactate) cut-off ≥ 7 was determined by (ROC) curve, with sensitivity of 80% and specificity of 78%. The area under the curve (AUC) of PELOD-2 score (with lactate) was 84.8% (95%CI 74.7 to 95.9%). A PELOD-2 score (without lactate) ≥ 7 was the most optimum cut off based on its Youden index, it had 70% of sensitivity and 80% of specificity.

Conclusion PELOD-2 score ≥ 7 , with or without lactate component is the optimal cut-off for predicting life-threatening organ dysfunction in pediatric sepsis. **[Paediatr Indones. 2020;60:227-32 ; DOI: 10.14238/pi60.4.2020.227-32]**.

Keywords: *PELOD-2 score, pediatric sepsis*

Sepsis is a leading cause of mortality and morbidity in infants and children. There is no gold standard for the diagnosis of sepsis, so the experts continue to refine the definition of sepsis.[1] *The First International Consensus of Sepsis* (Sepsis-1) defined sepsis as a systemic inflammatory response syndrome (SIRS) caused by suspected or proven infection. Severe sepsis was defined as sepsis associated with organ dysfunction, hypoperfusion, or hypotension.[2] This definition was later refined in 2001, with the acknowledgement of the predisposition, infection, response, and organ dysfunction (PIRO) system. Since then, several assessment systems have been developed to determine the severity of organ dysfunction.[3] Neither Sepsis-1 nor Sepsis-2 put forward specific parameters for children, so in 2005 a *Pediatric Sepsis Consensus Congress* (PSCC) was held to standardize the definition of sepsis in children by considering physiology and age-specific vital signs.[4]

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In 2016, the latest international consensus on sepsis (Sepsis-3) was issued; it defined sepsis as life-threatening organ dysfunction caused by immune dysregulation against infection. The consensus recommended that the sequential (sepsis-related) organ failure assessment (SOFA) score be used to evaluate life-threatening organ dysfunction.1 However, the SOFA score can only be applied to adults, due to the absence of special parameters for pediatric patients. Several studies have been conducted to adapt this score for children.[5] In the same year, the *Indonesian Pediatrics Society* (IPS) also issued a consensus statement on the diagnosis and management of pediatric sepsis. It refers to the definition of Sepsis-3, however, IPS recommended a different scoring system to assess organ dysfunction, the *Pediatric Logistic Organ Dysfunction-2* (PELOD-2) score. A PELOD-2 cut-off point of ≥ 11 was set as the criterion for predicting life-threatening organ dysfunction.[6]

A study at Cipto Mangunkusumo Hospital found that PELOD-2 score ≥ 10 was a predictor for life-threatening organ dysfunction for sepsis patients,[7] in contrast to a multicenter study in Europe who reported PELOD-2 score ≥ 8 as a cut-off point.[8] Differing cut-off points may have been due to differences in patient characteristics, facilities, and staff capability at each center. The aim of this study was to assess the validity of PELOD-2 scoring for predicting life-threatening organ dysfunction in children with sepsis.

Methods

This prospective cohort study was done in the Pediatric Intensive Care Unit, Mohammad Hoesin Hospital, Palembang, South Sumatera, from March to September 2019. All patients aged 1 month to 18 years who had organ dysfunction and two of the SIRS criteria, as well as infection according to the 2005 PSCC criteria[4] were included. Exclusion criteria were patients who died within the first 4 hours of PICU admission, or had multiple congenital anomalies, HIV infection, or neutropenia due to a medical condition other than SIRS.

A minimum required sample size of 62 patients would provide 80% power with a 95% level of significance. Six additional patients were included to allow for an estimated 10% drop out rate (patients transferred to other PICUs).

Subjects underwent physical and laboratory examinations. The PELOD-2 scores were calculated under supervision of a pediatric intensivist consultant. All subjects were followed until PICU treatment was completed. The outcomes were categorized as death in PICU or improvement (transferred to the ward).

Statistical analysis was performed using *SPSS software version 22.0*. Analysis by ROC curve was done to determine the optimal cut-off point of PELOD-2 score, both with and without lactate, for predicting life treathning organ dysfunction in pediatric sepsis. Regarding that not every health facilities were able to carry out lactate examination, we also aimed to revalidate the previously recommended cut-off point from the IPS consensus, which were score ≥11 for PELOD-2 with lactate and score ≥7 if lactate examination was excluded.

The Ethics Committee of Universitas Sriwijaya Medical School, Palembang, Indonesia approved this study. Informed consent was obtained from all parents.

Results

Of 70 patients who fulfilled the inclusion criteria, 66 enrolled in this study. Two children were excluded because of dengue shock syndrome (DSS) diagnosis, and two had incomplete data. Characteristics of subjects are reported in **Table 1**. The median age was 11 months (range 2-183) months, and 61% of subjects were male. Respiratory dysfunction occurred in 73% of subjects and the most common underlying infectious disease was pneumonia (41%).

The PICU mortality rate was 30%. **Table 2** shows the mortality distribution based PELOD-2 components. Glasgow coma scale (GCS), mean arterial pressure (MAP), lactate, and serum creatinine level had significant differences between the outcome groups.

The discrimination of PELOD-2 score was evaluated by calculating the AUC, by the result was 84.8% (95% CI: 74.7% to 95.9%). It means a moderate level of discrimination. An optimal cut-off point was determined by ROC analysis. This study found that PELOD-2 score (with lactate) ≥ 7 has

Table 1. Subjects' characteristics

Characteristics	Outcomes	
	Died in PICU (n=20)	Improved (n=46)
Age group, n		
1-11 months	11	22
12-23 months	1	7
24-59 months	1	9
60-143 months	3	4
≥144 months	4	4
Gender, n		
Male	13	27
Female	7	19
Nutritional status weight for height, n		
Severely wasted	5	6
Mild to moderately wasted	4	13
Normal	10	24
Overweight	1	3
GCS, n		
<11	14	19
≥11	6	27
Mean arterial pressure (MAP), n		
Hypotensive	13	9
Non-hypotensive	7	37
Pulse, n		
Normal	6	37
Tachycardic	12	9
Bradycardic	2	0
Temperature, n (%)		
< 36°C	0	1
36-37.9°C	12	23
> 37.9°C	8	22
Organ dysfunction*		
Cardiovascular	12	15
Respiratory	19	29
Neurologic	16	21
Hematologic	3	3
Renal	9	2
Hepatic	8	1
Underlying infection		
Pneumonia	10	17
Intracranial infection	4	15
Intraabdominal infection	1	5
Acute diarrhea with severe	1	4
dehydration	0	3
Surgical site infection	4	2
Others		
PICU length of stay, n		
< 48 hours	10	12
≥ 48 hours	10	34

*Subjects could suffer from more than one organ dysfunction

80% of sensitivity and 78% of specificity in predicting life threatening organ dysfunction (**Figure 1**). The AUC of the PELOD-2 score (without the lactate component) was 83.3% (95%CI 72.9 to 93.8) and a cut-off value ≥ 6 had 75% sensitivity and 72% specificity (**Figure 2**).

We observed that IPS's recommended cut-off point as a predictor of life-threatening organ dysfunction in children with sepsis (score ≥11, with lactate component) had 50% sensitivity and 98% specificity, with 91% positive predictive value (PPV) and 82% negative predictive value (NPV). A PELOD-2 score cut-off point ≥ 7 without lactate had 70% sensitivity and 80% specificity, with 61% PPV and 86% NPV.

Discussion

The median age of study subjects was 11 (range 2-183) months, with most subjects (50%) in the 1-11 month age group. Children in this age group have a higher risk of sepsis due to their immature immune system.[9] The majority of subjects (61%) were male. A previous study hypothesized that male sex hormones suppress the immune system, while female hormones actually trigger it. However, this explanation would not apply to infants far from puberty, so further investigation is needed.[10]

Pneumonia was the most common (41%) underlying infection found in this study. Endothelial damage in sepsis is caused by attachment and migration of pro-inflammatory cytokines to vasculature. This process must be preceded by an interaction between activated PMNs and endothelium. In certain organs, such as the lung, adhesion and migration through the endothelium can occur independently. Hence, the most common organ dysfunction in sepsis patients related to the respiratory system.[11]

The PELOD-2 scores were analyzed by ROC curve. The AUC was 84.8% (95%CI 74.7 to 95.9%), indicating a moderate level of discrimination in PELOD-2 score for predicting life-threatening organ dysfunction in pediatric sepsis. The IPS set a PELOD-2 score ≥ 11 for diagnosis and management of pediatric life-threatening organ dysfunction.5 We found that PELOD-2 score ≥ 11 had sensitivity of 50% and a specificity of 98%.

Mortality rates due to sepsis remain high. Late initiation of therapy because of late detection of sepsis leads to rapid deterioration, and eventual septic shock. To detect sepsis early, sensitive diagnostic criteria are

Table 2. Mortality distribution in association with each component of PELOD-2 score

PELOD-2 score criteria	Outcomes		Total	P value
	Died in PICU (n=20)	Improved (n=46)		
Neurologic				
GCS				0.032
<11	14	19	33	
≥11	6	27	33	
Pupillary reaction				0.133
Both reactive	17	44	61	
Both fixed	3	2	5	
Cardiovascular				
Lactatemia, mmol/L	16	45	61	0.012
<11	4	1	5	
≥11				
MAP				0.001
Hypotensive	13	9	22	
Non-hypotensive	7	37	44	
Renal				
Creatinine				0.000
Increased	9	2	11	
Normal	11	44	55	
Respiratory				
PaO2/FiO2				0.117
≥61	16	43	59	
≤60	4	3	7	
PaCO2, mmHg				0.198
≤58	15	40	55	
>58	5	6	11	
Invasive ventilation				0.064
Yes	17	29	46	
No	3	17	20	
Hematologic				
White blood count, x10^9/L				0.712
>2	19	46	65	
≤2	0	1	1	
Platelet count, x10^9/L				0.269
≥142	15	39	54	
< 142	5	7	12	

needed to avoid underdiagnoses.[12] The optimal cut-off point from our study was ≥ 7, with 80% sensitivity and 78% specificity. This cut-off point is more appropriate for predicting life-threatening organ dysfunction in pediatric sepsis than a value ≥ 11, which had only 50% sensitivity, based on our findings.

The positive predictive value of PELOD-2 score (with lactate) ≥11 was 91%, indicating that more than 90% of patients who had a score of ≥ 11 were expected to die. The PELOD-2 score ≥ 7 (without lactate) was able to predict mortality in 62% of sepsis patients, and predict no event of death by 90%, thus, using this cut-off to diagnose sepsis will increase early detection and provide better opportunities to prevent death.

A retrospective study at 9 PICUs in Europe reported an AUC of 91%, which had a high accuracy to assess the ability of the PELOD-2 score in discriminating outcomes of patients with infection. They found that the PELOD-2 score with lactate ≥ 8 had 85% sensitivity and a 88.4% specificity; a score ≥ 11 in subjects with hypotension and hyperlactatemia had a 97.83% sensitivity and 69.6%specificity.[8] The main difference in our study was the operational definition of infection: they established infection based on the clinical decision of the physician, while we used the PSCC (2005) sepsis criteria.[4] Median age, sex, and most organ dysfunction types were similar to those in our study.

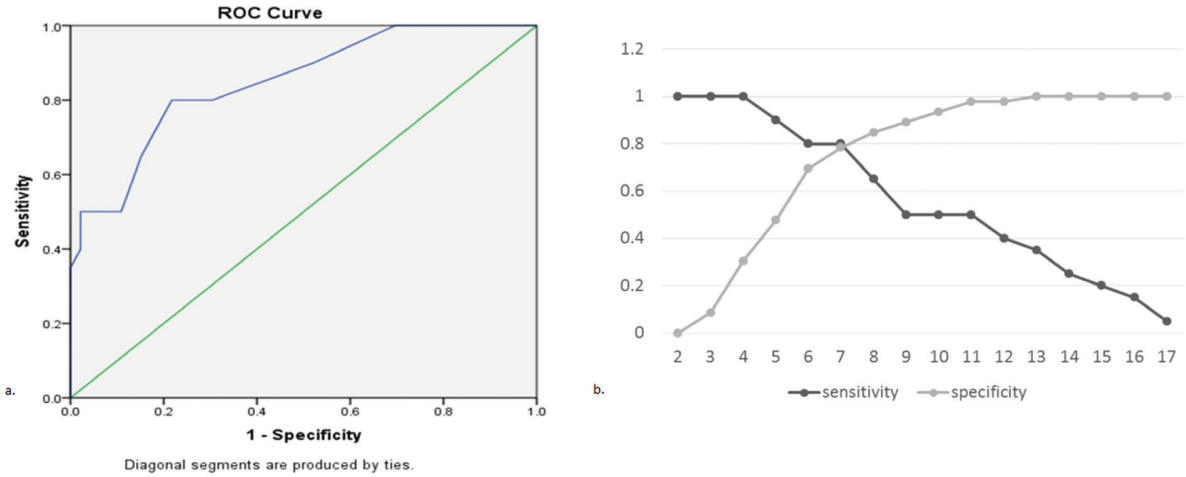

Figure 1. a) ROC curve for PELOD-2 score with lactate, b) cut-off point diagram

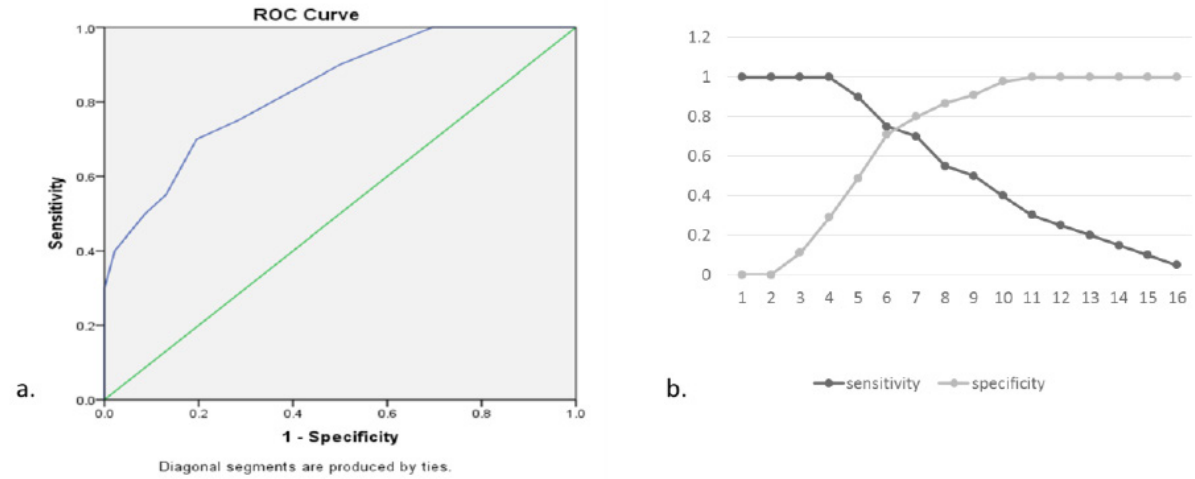

Figure 2. a) ROC curve for PELOD-2 score without lactate, b) cut-off point diagram

Suari[7] assessed the validity of PELOD-2 scores for predicting life-threatening organ dysfunction in pediatric sepsis patients at Cipto Mangunkusumo Hospital and noted an AUC of 85.5%, which was similar to ours. This study found the optimal cut-off point was ≥ 10, with 74% sensitivity and 76% specificity.[7] Subjects' characteristics of age, sex, and major organ dysfunction were similar to ours. However, the difference in cut-off points (≥10 *vs.* ≥7) may have been due to differences in sample size and the operational definition of infection used in subject selection. Supporting facilities and infrastructure as well as human resource capabilities can also affect patient outcomes, so the criteria used should be based on individual facilities.

Laboratory examinations such as lactate may not be available in areas with limited facilities, therefore, IPS also established the PELOD-2 cut-off score ≥7 without lactate.[5] The PELOD-2 score without lactate ≥7 had sensitivity of 70% and specificity of 80%. The ROC curve analysis revealed that PELOD-2 cut-off point ≥6 without lactate had sensitivity of 75% and specificity of 72%. Analysis with ROC assesses the optimal cut-off value based on a balance between sensitivity and specificity. The PELOD-2 cut-off values of ≥6 and ≥7 yielded similar results. Another method to determine the best cut-off value is the Youden index. A Youden index close to 1 or higher

is interpreted as optimal.[12] The Youden index for PELOD-2 score ≥ 7 was 0.504, while that for ≥ 6 was 0.467, indicating that the cut-off score ≥ 7 (without lactate) is more optimal to be used for predicting life-threatening organ dysfunction in pediatric sepsis.

We included only patients with sepsis at PICU admission, while patients who got sepsis during PICU treatment were not studied, so our results might not be applicable to overall sepsis in the PICU. We also validated for PELOD-2 score without the lactate component, for the purpose of use in hospitals with limited facilities, not for a center like our hospital. Differences in facilities and staff capability may also affect patient outcomes.

In conclusion, PELOD-2 score ≥ 7, with or without the lactate component, is the optimal cut-off point to predict life-threatening organ dysfunction in pediatric sepsis. Multicenter revalidation is needed to determine the most optimal cut-off point for general use in Indonesia.

Conflict of Interest

None declared.

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Original Article

Knee height and knee height/height ratio of healthy schoolchildren

Annang Giri Moelyo, Andre Christiawan Susanto, Bella Monika Rajagukguk, Jonathan Billy Christian Tjiayadi

Abstract

Background Knee height (KH) is rarely used to estimate stature in children, although its measurement might have benefit because not influenced by some musculoskeletal disorder in spinal region. Knee height and knee height/height ratio are typical in children due to different in pubertal timing of each child.

Objective To derive a formula to estimate body height using knee height and to analyze the patterns of knee height and knee height/height ratio of healthy schoolchildren.

Methods This cross-sectional study involved healthy children in one elementary school and one junior high school in Surakarta, Central Java. Demographic data were collected (sex, age, and ethnicity). All anthropometric measurements (height, weight, sitting height, and knee height) were taken three times, and their means were calculated. Linear regression analysis was used to compare height from knee height and sitting height. Non-parametric analysis through locally weighted scatterplot smoothing (LOWESS) was used to analyze the growth patterns of knee height, knee height/height ratio, and sitting height/height ratio.

Results There were 633 children (328 boys and 305 girls) in this study. The formulas for the estimation of height were as follows: for boys, 2.40 × KH (cm) + 1.36 × age (years) + 20.31; and for girls, 2.48 × KH (cm) + 1.15 × age (years) + 19.58 (adjusted R2=0.97). Knee height increased earlier than sitting height in both boys and girls during childhood to adolescent period. Boys had a longer period of knee height increment than girls.

Conclusion Knee height may be a useful alternative to estimate height in children. Knee height increases faster than height and sitting height in both boys and girls. **[Paediatr Indones. 2020;60:233-8; DOI: 10.14238/pi60.5.2020.233-8]**.

Keywords: *knee height; knee height/height ratio; schoolchildren*

Shorter leg length, including knee height, has been associated with risks of metabolic syndrome (obesity, coronary heart disease, and diabetes), liver dysfunction, and certain cancers in adulthood.[1] Leg length is also an indicator of environmental quality for growth during childhood.[1]

Knee height, a part of leg length, has been used as a measurement to estimate stature, especially in elderly or critically ill patients; other such measurements include leg length, sitting height, arm span, and upper-to-lower segment ratio.[2-4] Knee height or leg length assessment in children changes with age, especially during puberty. In clinical practice, knee height is rarely used to estimate body height in children. However, because of difficulties in measuring the body height in some conditions such as scoliosis and cerebral palsy, children's body height could be estimated using other measurements, such as sitting height or knee height.[5,6]

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We aimed to develop a formula using knee height to predict height in children, and compared that formula to equations using sitting height. We also analyzed knee height and knee height/height ratio patterns among schoolchildren in Surakarta, Central Java, Indonesia.

Methods

This study was conducted on healthy children in one elementary school and one junior high school from September 2017 to September 2018 in Surakarta, Central Java, Indonesia. Ethical approval was obtained from the Health Research Committee, Dr. Moewardi General Hospital, Universitas Sebelas Maret Medical School. Parental written informed consent was obtained. Students who were present at their school during the study periods were included.

Subjects' demographic data were collected (gender, birth date, and parents' ethnicity) from school records. We interviewed the children for any incomplete data. If both parents were Javanese or Chinese, we categorized the children as Javanese or Chinese, respectively; "other" was used for other or mixed ethnicities.

Anthropometric measurements (height, weight, sitting height, and knee height) were taken three times by trained personnel, then the means were calculated. Height and sitting height were measured using a wall stadiometer (*Stature Meter 2M GEA*) to the nearest 0.1 cm with the child facing the examiner. Knee height was measured to the nearest 0.1 cm on the right lower leg using a knee height caliper belonging to the Department of Child Health, Universitas Sebelas Maret Medical School, with the child sitting upright in a chair facing forward and hands at sides, and both the knee and ankle at 90°.1 Bodyweight was measured using a digital scale (*Seca Clara 803*, Germany) to the nearest 0.1 kg. The sitting height/height ratio was calculated by dividing the sitting height by total height; the knee height/height ratio was calculated by dividing knee height by total height.

The *WHO* classification was used to define overweight/obesity (body mass index > 2 SD), short stature (height-for-age Z-score (HAZ) < -2 SD), normostature (-2≤HAZ≤2), and tall stature (HAZ>2 SD). Non-parametric analysis through locally weighted scatterplot smoothing (*LOWESS*) was used to analyze growth increment and growth patterns of height, knee height, and knee height/height ratio. All statistics were analyzed using Stata software 14.0.

Results

We recruited 633 children (328 boys, 305 girls) into this study. Their characteristics are described in **Table 1**. The equations for height estimation from knee height and sitting height for boys and girls are described in **Table 2**.

Table 3 shows the height increment and KH increment in boys and girls. The mean height increment > 6 cm/year was seen in boys aged 9-11 years and girls aged 9-10 years. The mean knee height increment > 2 cm/year was seen in boys aged 7-10 years and girls aged 9-10 years. The mean knee heights

Table 1. Baseline characteristics of subjects

Characteristics	Males (n=328)	Females (n=305)	Total (N=633)
Ethnicity, n(%)			
Chinese	16 (4.9)	17 (5.6)	33 (5.2)
Javanese	246 (75.0)	228 (74.8)	474 (74.9)
Other	66 (20.1)	60 (19.7)	126 (19.9)
Age, years			
Mean (SD)	11.1 (3.0)	10.4 (2.6)	10.8 (2.8)
Range	5.8-16.4	5.8-16.6	5.8-16.6
Overweight-obese, n (%)	77 (23.45)	40 (13.1)	117 (18.5)
Stature, n (%)			
Short	23 (7)	35 (12)	58 (9)
Normal	302 (92)	265 (86)	567 (90)
Tall	3 (1)	5 (2)	8 (1)

Table 2. Formulas using KH and sitting height to predict body height

Variables	Male		Female	
	Equation	Adj. R^2	Equation	Adj. R^2
Knee height	Height = 2.40*knee (cm) + 1.36*age (yrs) + 20.31	0.97	Height = 2.48*knee (cm) + 1.15*age (yrs) + 19.58	0.97
Sitting-height	Height = 1.34*sitting (cm) + 2.39*age (yrs) +15.95	0.95	Height = 1.60*sitting (cm) + 1.27*age (yrs) + 7.25	0.95

Table 3. Mean height, knee height, and their increment by yearly age

Boys						Girls					
Age	n	Height, cm	Δ height, cm	KH, cm	Δ KH, cm	Age	n	Height, cm	Δ height, cm	KH, cm	Δ KH, cm
5-<6	3	110.69	~	33.47	~	5-<6	4	112.94	-	34.37	-
6-<7	34	114.27	+3.58	34.78	+1.31	6-<7	33	115.34	+2.41	35.23	+0.86
7-<8	29	119.99	+5.72	36.94	+2.16	7-<8	34	119.81	+4.47	36.83	+1.60
8-<9	32	125.52	+5.53	39.01	+2.07	8-<9	29	124.65	+4.84	38.58	+1.74
9-<10	36	131.61	+6.10	41.23	+2.21	9-<10	35	131.61	+6.95	41.00	+2.42
10-<11	30	137.93	+6.32	43.42	+2.20	10-<11	39	137.75	+6.14	43.04	+2.04
11-<12	19	143.97	+6.05	45.35	+1.93	11-<12	44	142.43	+4.68	44.37	+1.33
12-<13	35	149.81	+5.84	47.08	+1.73	12-<13	16	147.92	+5.49	45.92	+1.56
13-<14	36	155.79	+5.98	48.75	+1.66	13-<14	34	151.19	+3.27	46.72	+0.80
14-<15	47	160.66	+4.87	49.93	+1.19	14-<15	30	153.55	+2.35	47.19	+0.48
15-<16	19	164.26	+3.59	50.54	+0.60	15-<16	6	155.13	+1.58	47.47	+0.27
16-17	8	166.90	+2.64	50.82	+0.28	16-17	1	156.88	+1.76	47.74	+0.27

among short, normal, and tall stature groups were significantly different (**Table 4**).

Table 4. Mean knee heights among subjects with short, normal, and tall stature

Variables	n (%)	Mean age* (SD)	Mean KH** (SD)
Short stature	58 (9)	10.34 (2.89)	38.74 (4.90)
Normal stature	567 (90)	10.79 (2.82)	43.45 (5.50)
Tall stature	8 (1)	11.73 (2.38)	50.52 (4.54)

*Anova test (P>0.05); **Anova test (P<0.05)

Figure 1 describes the patterns of height, knee height/height ratio, sitting height/height ratio in boys and girls according to age. Knee height/height ratio increased until peaking around age 11 for both boys and girls. However, sitting height/height ratio patterns decreased during the increment of knee height/height ratio for boys and girls aged 6-11 years. **Figure 2** illustrates the patterns of knee height/height ratio by age in short stature and overweight-obese children compared to normal stature and normoweight subjects. The patterns of short stature and/or obese were similar to normal subjects, for boys and girls.

Figure 3 shows the patterns of knee height/height ratio in children stratified by ethnicity, according to age and sex. The Chinese, and also others' ethnicity, growth patterns were similar to Javanese one.

Discussion

Knee height measurement is rarely used in children, although leg length is associated with the quality of the environment in children's growth. Our study demonstrated a positive correlation between knee height and body height. The formula of knee height were concordant to sitting height (adjusted R^2 were 0.97 *vs.* 0.95, respectively). The formulas of knee height were different with an adult study in Indonesia {height = (1.647 x kneeheight) + 80.08 (male), height = (1.807 x kneeheight) + 66.54 (female)}.[4] The adjusted R^2 of knee height (male/female) in our pediatric study was larger than that in the study involving elderly individuals (0.97/0.97 *vs.* 0.512/0.579, respectively).[4] Hence, this formula can be used to estimate body height in schoolchildren, although the knee height/height ratio is dynamic by age. We still need to verify the accuracy of the

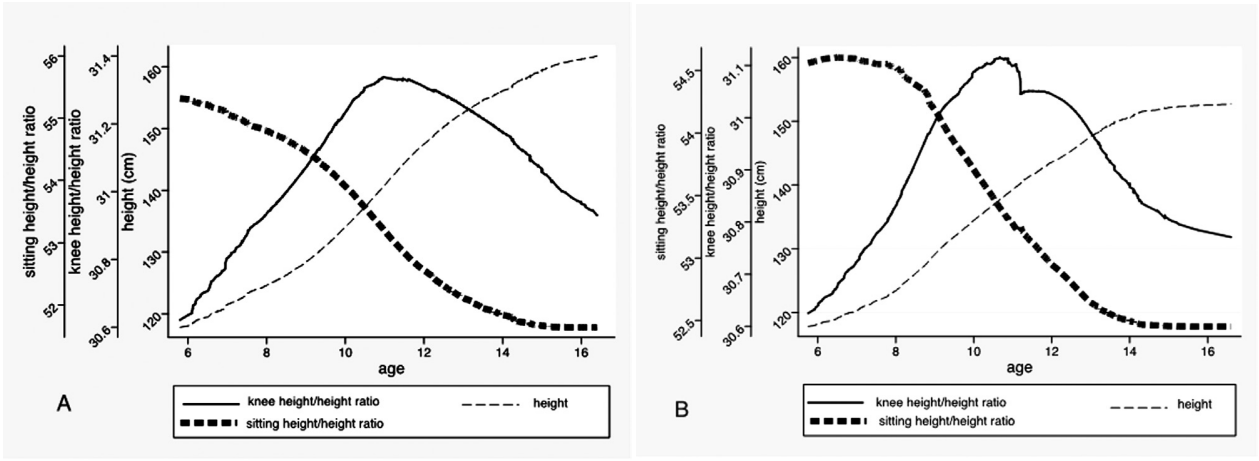

Figure 1. Patterns of height, knee height/height ratio, sitting height/height ratio of subjects [knee height/height ratio = (knee height/body height x 100); sitting height/height ratio=(sitting height/body height x 100)]. (A) Boys (B) Girls

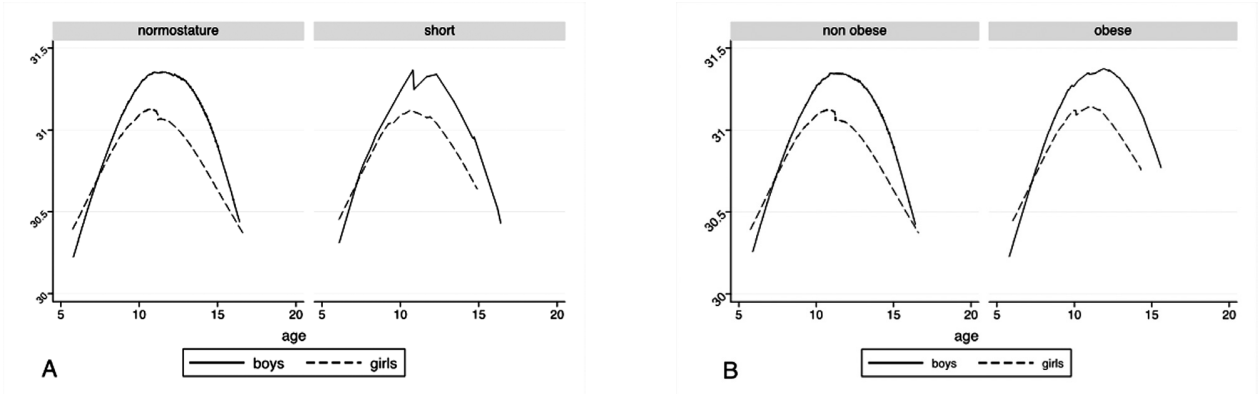

Figure 2. Patterns of knee height/height ratio in short stature and overweight-obese subjects by age and sex; (A) stature and (B) weight status

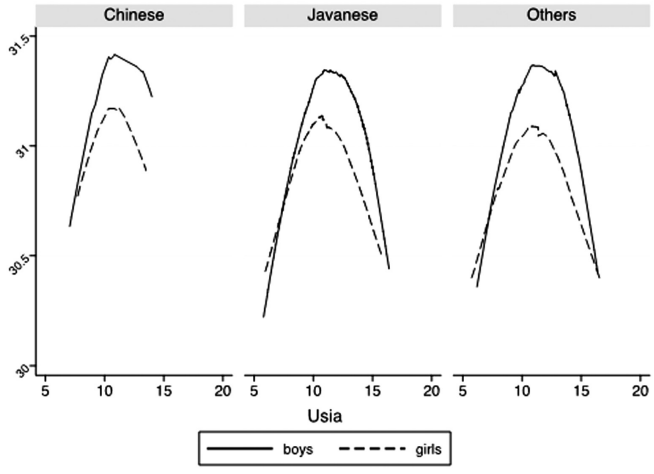

Figure 3. Patterns of knee height/height ratio according to ethnicity, age, and sex

equations to estimate body height compared to actual height in other schoolchildren.

The knee height in schoolchildren in our study was comparable to that in a 2015 Jakarta study.[5] A previous study recommended re-evaluating the Indonesian National Standard for elementary school furniture because of the mismatch between standard and anthropometric data. We also noted this mismatch, hence, we agree with their recommendation.[5] For this purpose, a much larger dataset on knee height measurements in schoolchildren will be needed.

The human body has characteristically longer legs compared to other species.[1,7] The differences between the lowest and highest values of the knee height/height ratio in our study were not large (30.2-31.5 in boys and 30.4-31.2 in girls). A previous study showed a similar ratio in the same age range, but the peak knee height/height ratio in our study was smaller (31.5/31.2 *vs.* 32.3, respectively).[6] The increase in the knee height/height ratio with age was concurrent with the decrease in sitting height/height ratio with age (**Figure 1**). We noted that knee height grew faster than sitting height (i.e., the lower segment grew faster than the upper segment of the body). More precise data are needed on puberty or maturity in schoolchildren to analyze for an association between early growth of knee height and pubertal stage.

The patterns of the knee height/height ratio between boys and girls were different. Knee height increment and knee height/height ratio patterns in girls were steeper and narrower than those in boys, thus lowering the peak of the ratio in girls. Our previous study on the sitting height/height ratio in adolescents showed similar values between boys and girls.[8] The knee height of children with short stature was smaller than that of children with normal stature, but there were no differences in the patterns of knee height/height ratio with respect to stature in our subjects. The peak knee height/height ratios in children with short or normal stature were similar. Thus, people with short stature had short legs, but their body proportion between upper and lower segment remained unchanged. Overweight/obesity did not affect the knee height/height ratio patterns, even though it is evident that obese children mature earlier. Additional data on the patterns of knee height growth in children less than 5 years of age are required to analyze for conditions such as short stature and obesity.

Regarding the influence of ethnicity, Chinese subjects had the same patterns of knee height/height ratio as Javanese children, who comprised the majority of our cohort. These results were in agreement with those of our previous study on sitting height/height ratio and another study involving an Asian population with similar proportions.[8,9]

There were some limitations to our study. Since we conducted a study of urban schoolchildren from one elementary and one junior high school in Surakarta, Central Java, our sample might not be representative of all schoolchildren in Indonesia. Follow-up studies on the same cohort are needed to elucidate the change in patterns of height, knee height, and knee height/height ratio with respect to the growth of the schoolchildren. Future studies should analyze for pubertal or maturity factors. The Preece-Baines growth model is useful to assess the peak height or knee height velocity. However, this model needs longitudinal data and does not work well with cross-sectional data, which was used in our study.[10,11] To comment on whether short legs were associated with the quality of the environment during growth, data on family socioeconomic backgrounds are needed, which were unavailable in our study.[7] We also did not assess the cause of short stature as to whether it was a normal variation (e.g., familial short stature, constitutional delay of growth) or an underlying pathology (caused by inadequate nutrition or infection). Additional study is needed to further analyze for links among environment quality, knee height, and body height. Measurement of knee height, compared to sitting height, was more difficult to conduct in schoolchildren.[6] For clinical practice, we can use knee height to estimate body height whenever sitting height cannot be measured.

In conclusion, knee height may be used to estimate height in children. Knee height increases faster than height and sitting height during puberty in male and female children. Boys have a longer period of knee height increment than girls. Short stature, overweight/obesity, and ethnicity do not affect the knee height patterns.

Conflict of interest

None declared.

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Original Article

Age at menarche and body fat in adolescent girls

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Abstract

Background Menarcheal age is important in adolescent girls due to its associations with health outcomes at adulthood. Modifiable factors that may influence menarcheal age include body fat mass and fat distribution.

Objective To investigate possible correlations between body fat mass and fat distribution with age at menarche in adolescent girls.

Methods This study was a cross-sectional study on 32 girls aged 10-15 years in Central Jakarta, who experienced menarche within the time period of July to September 2019. Data on menarcheal age was collected by recall. Body fat mass and distribution were calculated using anthropometric measurements and bioelectrical impedance analyzer (BIA) results.

Results The mean age of study subjects was 12.06 (SD 0.82) years and the mean age at menarche was 11.91 (SD 0.83) years. Correlation tests revealed a moderate negative correlation between body mass index-for-age and menarcheal age (r=-0.45; P=0.01) and weak negative correlation between waist-height ratio and menarcheal age (r=-0.37; P=0.03).

Conclusion Menarcheal age is correlated with body mass index-for-age and waist-height ratio. However, no significant correlations between menarcheal age and body fat mass or distribution are found. **[Paediatr Indones. 2020;60:269-76; DOI: 10.14238/pi60.5.2020.269-76]**.

Keywords: *menarcheal age; age at menarche; body fat mass; body fat distribution*

Menarche is defined as the first menstrual period experienced by an adolescent girl. It is considered to be the most recognizable feature of puberty in girls, generally in the mean age of 12.88 (SD 1.27) years.[1,2] Age at menarche is important to the overall clinical patient history, because it is known to influence health in adulthood. Girls with younger menarcheal age (<12 years) tend to have higher blood pressure, glucose intolerance, cardiovascular disease, and higher mortality due to cancer.[3,4] Older menarcheal age (>14 years) is associated with lower bone density which increases the risk for osteoporosis.[5] *The 2010 Indonesian Basic Health Research Report* showed that 22.5% of Indonesian girls underwent early menarche and 24.3% underwent late menarche.[6] Throughout the world, especially in low-to-middle income countries, menarche is associated with taboos and sanitation problems, which can lead to missed school

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days, isolation, and gender inequality, as well as barriers to adequate sexual and reproductive health education. Recognizing and addressing the problems related to menstruation can promote attainment of the *Sustainable Development Goals*, including, but not limited to, SDG 3 (Good Health), SDG 4 (Quality Education), and SDG 5 (Gender Equality).[7]

The advancement of socioeconomic and health conditions in the 20th century led to a shift to earlier menarcheal age. This shift is known as a secular trend, observed worldwide,[8-10] including in Indonesia, a low-middle income country with diverse socioeconomic and environmental conditions. A systematic review described the secular trend in menarcheal age in Indonesia in 1970-2010, which decreased from 14.43 years to 13.63 years, and was predicted to continue to decrease by 0.0245 years (8-9 days) per year.[11]

Age at menarche is influenced by genetic and non-genetic factors, including body fat mass. Body fat can affect menarcheal age through leptin, a protein produced by adipocytes, which increases with the amount of body fat. Leptin stimulates the hypothalamus to secrete gonadotropin-releasing hormone (GnRH), which in turn stimulates the pituitary gland-ovarium axis and accelerates puberty. Fat distribution also plays a role in menarcheal age; leptin levels have stronger associations with gluteofemoral fat compared to upper body fat.[3]

Body fat can be measured through anthropometric measurements, skinfold thickness (SFT), or body composition measurement tools. Anthropometric and SFT measurements are easier and less expensive to perform, but may be affected by intraobserver and interobserver errors, due to their subjective nature. Body composition measurement tools and machines have better accuracy, but are more expensive and their usage is mostly limited to research purposes.[12,13] In addition, bioelectrical impedance analysis (BIA), a body composition measurement tool to assess fat distribution, is accurate, easy to use, comfortable for subjects, and relatively affordable.[13]

Body fat and fat distribution are determining factors of menarcheal age that can be modified through lifestyle changes. Investigating the influence of these factors on menarcheal age can help improve morbidity risk due to early or late menarche, in line with the third goal of the *Sustainable Development Goals* (SDGs) to reduce mortality due to non-communicable diseases (type 2 diabetes mellitus, cardiovascular diseases, and cancer) through prevention.[14] This study was aimed to investigate possible correlations between body fat mass and fat distribution with age at menarche in adolescent girls.

Methods

This cross-sectional study was performed in July-September 2019 in Central Jakarta, Indonesia. We visited primary and junior high schools closest to the Universitas Indonesia Medical School, which was the location of measurement. We recruited 32 girls aged 10-15 years who experienced menarche in the last 3 months from 10 primary schools (SDN Cikini 01, SDN Cikini 02, SDN Paseban 03, SDN Paseban 05, SDN Paseban 07, SDN Kramat 06 pagi, SDN Kramat 08 pagi, SDN Johar Baru 01, SDN Pegangsaan 01, and SDN Kenari 07) and seven junior high schools (SLTPN 2 Jakarta, SLTPN 8 Jakarta, SLTPN 28 Jakarta, SLTPN 71 Jakarta, SLTPN 216 Jakarta, SLTP Muhammadiyah 3, and SLTP Muhammadiyah 16).

Our inclusion criteria were girls aged 10-15 years who experienced menarche in the previous 3 months, had no changes in body weight greater than 5% in the previous 3 months, signed an informed assent form, and whose parents or guardians signed informed consent forms. We excluded girls who had precocious puberty, were absent during data collection, or had other conditions that may affect anthropometric measurements (organomegaly, edema, scoliosis, musculoskeletal problems, and other syndromes or congenital defects with growth disorders).

Subjects were asked to fill questionnaires on menarcheal age and socioeconomic characteristics. Menarcheal age was obtained through recall, by calculating the time period between the day the subject menstruated for the first time and her date of birth (in years and months with two decimal points). Parental education level was divided into three groups: low (primary school or the equivalent), intermediate (junior high, high school, or the equivalent), and high (diploma, bachelor's degree, master's degree, or the equivalent). Parental income was divided into two groups based on the Jakarta Regional Minimum Wage in 2019 (IDR 3,900,000/month or around USD 247/month): low (below minimum wage) and high (equal

to or above minimum wage).

The investigating team and other trained staff measured body fat mass and distribution using anthropometric measurements and BIA at IMERI, Universitas Indonesia Medical School. The anthropometric measurements were body mass index (BMI), waist circumference, hip circumference, waist-hip ratio, and waist-height ratio. The BMI was calculated by dividing weight (kg) by height-squared (m2), stated in kg/m^2. We measured BMI-for-age using the CDC BMI-for-age chart, stated in *z*-score with 2 decimal points.[15] During measurements, subjects wore their school uniforms and no shoes. Body weight and body fat mass were measured once each using a *Tanita MC 780 BIA* (*Tanita Corporation*, Tokyo, Japan), with 0.1 kg precision. Body height was measured twice using a stadiometer with 0.1 cm precision and an mean of the two measurements was recorded.

Waist circumference was measured with the subject standing upright and the measuring tape placed between the lowest rib and the iliac crest; a mean of two measurements at the end of expiration was recorded (cm). Hip circumference was measured with the subject standing upright and the measuring tape placed on the largest circumference of the hip area; a mean of two measurements was recorded (cm). The waist-hip ratio was calculated by dividing waist circumference by hip circumference. The waist-height ratio was calculated by dividing waist circumference by body height.

The BIA measurements obtained for this study included total body fat mass (TBFM), body fat mass of the upper extremities (BFMUE), body fat mass of the lower extremities (BFMLE), body fat mass of the trunk (BFMT), ratio of body fat mass of the extremities and the trunk (BFMET ratio), percentage of total body fat (%BF), percentage of fat in the upper extremities (%BFUE), percentage of fat in the lower extremities (%BFLE), and percentage of fat in the trunk (%BFT). Measurements were done once per subject.

The data were analyzed using *SPSS version 22 for Windows*. Descriptive data for categorical variables (name of school, parental education and socioeconomic levels) are stated in frequency (n) and percentage (%). Descriptive data for numerical variables (age, menarcheal age, weight-for-age, height-for-age, BMI, BMI-for-age, waist circumference, hip circumference, waist-hip ratio, waist-height ratio, TBFM, BFMUE, BFMLE, BFMT, BFMET ratio, %BF, %BFUE, %BFLE, and %BFT) were stated as mean, median, interquartile range, and standard deviation. Shapiro-Wilk test was done to assess data normality prior to hypothesis testing. Correlations between body fat measurements and menarcheal age were analyzed by Pearson's or Spearman's tests. Results with P values <0.05 were considered to be statistically significant. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia.

Results

We recruited 102 girls who experienced menarche in the last three months, out of 1,498 girls enrolled in 10 primary schools and seven junior high schools. Parental informed consent and subject informed assent were requested from the 102 girls, however, only 32 subjects' parents consented to participation.

Most subjects were categorized as middle-to-low socioeconomic class. Subjects' mean age was 12.06 (SD 0.82) years and their mean age at menarche was 11.91 (SD 0.83) years. The characteristics of subjects are described in **Table 1**.

We analyzed for potential body fat mass and distribution correlations with menarcheal age. Pearson's correlation test was used for BMI, BMI-for-age, waist circumference, hip circumference, waist-hip ratio, and %BFUE. Spearman's correlation test was used for waist-height ratio, TBFM, BFMUE, BFMLE, BFMT, BFMET ratio, %BF, %BFLE, and %BFT. Two variables had statistically significant correlations with menarcheal age: BMI-for-age (moderate negative correlation; r=-0.45; P=0.01) and waist-height ratio (weak negative correlation; r=-0.37; P=0.03) (**Table 2**). The scatter plots for the correlation tests are shown in **Figure 2**.

Discussion

A multicenter study in Indonesia on menarcheal age in 1992-1995 showed the mean menarcheal age in Indonesia to be 12.96 years, with a mean BMI of 19.17 kg/m^2. The mean menarcheal age in Jakarta was 12.89 years, with a mean BMI of 19.54 kg/m^2.[10] A Surakarta, Central Java study on 835 healthy

Table 1. Characteristics of study subjects

Characteristics	Total (N = 32)
School, n (%)	
Primary school	18 (56)
Junior high school	14 (44)
Paternal education level, n (%)	
Low	7 (22)
Intermediate	17 (53)
High	8 (25)
Maternal education level, n (%)	
Low	2 (6)
Intermediate	26 (81)
High	4 (13)
Parental income, n (%)	
Low	21 (66)
High	11 (34)
Median body weight (IQR), kg	41.25 (9,88)
Mean body weight-for-age (SD), Z-score*	0.01 (1.09)
Mean body height (SD), cm	149.95 (4.72)
Mean body height-for-age (SD), (Z-score)*	-0.51 (0.93)
Mean BMI (SD), kg/m2	19.92 (3.56)
Mean BMI-for-age (SD), Z-score*	0.34 (0.98)
Mean waist circumference (SD), cm	68.54 (7.94)
Mean hip circumference (SD), cm	83.25 (6.01
Mean waist-hip ratio (SD)	0.82 (0.06)
Median waist-height ratio (IQR)	0.43 (0.06)
Median total body fat mass (IQR), kg	9.9 (5.65)
Median body fat mass of the upper extremities (IQR), kg	0.7 (0.53)
Median body fat mass of the lower extremities (IQR), kg	4.8 (2.15)
Median body fat mass of the trunk (IQR), kg	4.55 (3)
Median ratio of the body fat mass in the extremities and trunk (IQR)	1.2 (0.32)
Median body fat percentage (IQR), %	23.65 (7.32)
Mean percentage of fat in the upper extremities (SD), %	7.26 (1)
Median percentage of fat in the lower extremities (IQR), %	46.98 (6.26)
Median percentage of fat in the trunk (IQR), %	45.34 (6.59)

SD=standard deviation; IQR=interquartile range; *CDC growth chart (2000)

Table 2. Correlation analyses of body fat mass and fat distribution measurements with menarcheal age

Characteristics	Menarcheal age	
	R	P value
BMI, kg/m^2	-0.28	0.11[a]
BMI-for-age, Z-scores	-0.45	0.01[a]
Waist circumference, cm	-0.32	0.06[a]
Hip circumference, cm	-0.21	0.23[a]
Waist-hip ratio	-0.29	0.10[a]
Waist-height ratio	-0.37	0.03[b]
Total body fat mass, kg	-0.06	0.70[b]
Body fat mass of the upper extremities, kg	-0.12	0.48[b]
Body fat mass of the lower extremities, kg	-0.19	0.28[b]
Body fat mass of the trunk, kg	-0.03	0.87[b]
Body fat mass of the extremities and the trunk ratio	-0.07	0.68[b]
Body fat percentage,%	-0.04	0.78[b]
Percentage of body fat in the upper extremities, %	-0.24	0.17[a]
Percentage of body fat in the lower extremities, %	-0.01	0.94[b]
Percentage of body fat in the trunk, %	0.04	0.82[b]

a=Pearson's correlation test; b=Spearman's correlation test

adolescent girls in 2018 reported a mean age at menarche of 12 years, with a mean BMI of 20.7 kg/m^2. The majority (99.04%) of subjects had menarche by the age of 14 years.[16] *The 2010 Indonesian Basic Health Research Survey* reported the mean menarcheal age in Indonesia was 12.74 (SD 1.19) years, based on a survey on 1,418 girls.[17] In 2011, a study on 128 primary and junior high school students in Jakarta and two suburban settlements near Jakarta found a mean menarcheal age of 12.18 (SD 0.91) years, with a mean BMI OF 18.87 (SD 2.89) kg/m^2.[18] Another study in an Islamic school (equivalent to junior high school) in 2013 found a mean menarcheal age of 11.68 (SD 0.71), with a mean BMI of 20.05 (SD 4.23) kg/m^2.[19] These studies, done in different years, show a secular trend of decreasing menarcheal age in Indonesia, although not as precisely as previously predicted by Wahab *et al.*,[11] which was approximately 0.0245 years (8-9 days) per year.

The mean menarcheal age in our subjects was 11.91 (SD 0.83) years, with a mean BMI of 19.92 (SD 3.56) kg/m^2. Comparing study results is challenging due to differences in inclusion criterion age cut-offs. We used an age cut-off of 10-15 years, excluding girls who experienced menarche outside the age range. We also excluded girls with precocious puberty (puberty before 8 years of age).

We found menarche to happen mostly (75%) in adolescents with normal BMI. The Frisch and Revelle hypothesis stated that menarche in an adolescent

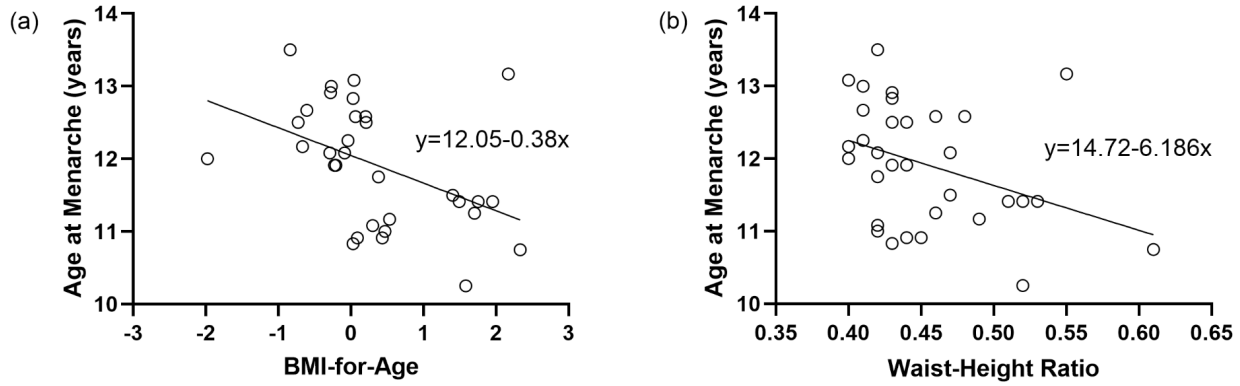

Figure 1. (a) Correlation between age at menarche and BMI-for-age, (b) Correlation between age at menarche and waist-height ratio.

girl can occur when her body weight reaches a minimum of 48 kg or 17% body fat percentage.[20] Our subjects median weight was 41.25 (range 29.8-68.6) kg and median body fat percentage was 23.65 (range 14.1-47.1)%. Our findings suggest that body weight and body fat percentage are not the only determining factors of menarche. Other factors such as fat distribution, nutrition, environment, and genetics may also play a role.

Correlation tests revealed statistically significant relationships between menarcheal age and BMI-for-age, as well as waist-height ratio. We found a moderate negative correlation between BMI-for-age and menarcheal age; girls with higher BMI-for-age Z-scores tended to undergo menarche earlier. Our finding was consistent with a study in Spain on girls aged 13-16 years, which found BMI-for-age to be weakly correlated with menarcheal age ($r=-0.34$).[21] Other Indonesian studies on the relationship between menarcheal age and BMI, not BMI-for-age, found varying degrees of negative correlations ($r=-0.31$, $r=-0.33$, and $r=-0.98$, respectively).[18,22,23]

We measured waist circumference, waist-hip ratio, and waist-height ratio to assess abdominal obesity, and found a weak correlation between waist-height ratio and menarcheal age ($r=-0.37$). A study in Tanzania found no statistically significant differences between waist-hip ratio and waist-height ratio of pre-menarche and post-menarche girls.20 In addition, another study found a strong positive correlation between waist-hip ratio and menarcheal age ($r=0.95$).[23] This difference may have been due to different study methods, especially with regards to sample size, age limitation of study subjects at recruitment, baseline characteristics of subjects, and the time period between data collection and menarche.

Body fat mass and fat distribution was measured by BIA. We found no correlation between body fat percentage and menarcheal age, which contradicted previous studies which found significant correlations of varying strengths. Sterling investigated body fat percentage measured by *Tanita BIA* and found a positive correlation coefficient of 0.09 for the relationship between body fat percentage and menarcheal age in 251 adolescent girls aged 12-19 years in California and Michigan, USA.[24] A prospective cohort study on 156 pre-menarche girls in primary school grades 4-6 found a positive correlation coefficient of 0.28 for such a relationship.[22] In addition, a strong positive correlation between body fat percentage and menarcheal age was reported by a previous study on 44 girls in Padang, West Sumatera, Indonesia, who experienced menarche within 12 months of the study ($r=0.97$).[23]

We found no significant correlation between segmental fat distribution and menarcheal age. Previous studies on menarcheal age generally used skinfold thickness or anthropometric measurements to assess fat distribution, with limited use of BIA. A previous study used the *Third National Health and Nutrition Examination Survey* (NHANES III) data

of 10-14-year-old girls and found that increased hip circumference was positively correlated with menarche (OR 1.22; 95%CI 1.17 to 1.26; P<0.01), while the increase of waist circumference and triceps skinfold thickness was negatively associated with menarche [(OR 0.93; 95%CI 0.90 to 0.96; P<0.01) and (OR 0.91; 95%CI 0.88 to 0.94; P<0.01), respectively]. Menarche occurred in girls with low total body fat mass, but adequate gluteofemoral fat mass.[25] A previous study investigated subcutaneous fat distribution using skinfold thickness in pre- and post-menarche girls in India, and found no difference in the ratio of subcutaneous fat in the trunk and extremities in the two groups. Post-menarche girls had more fat mass in the upper trunk and upper extremities.[26]

Menarcheal age is influenced by genetic and non-genetic factors, such as environment, socioeconomic status, nutrition, and body fat.[3] Our study focused on how body fat affects menarcheal age, because the modifiable nature of body fat and body fat levels are easy to monitor. The relationship between body fat and puberty starts in infancy. Formula-feeding has been associated with rapid weight gain after birth and earlier menarche. This rapid weight gain in infancy and childhood may disrupt hormonal balance and growth velocity, as seen in the increase of androgen levels at 8 years of age. Insulin resistance and peripheral hyperinsulinemia are common in obese children, with effects on several organs (adrenal glands, liver, ovarium, and adipocytes), leading to increased bioavailability of sex hormones. This increase in sex hormones may activate the hypothalamus-pituitary axis to start puberty earlier.[27] A longitudinal study showed that high BMI at 3 years of age in conjunction with fast BMI increase at 3-6 years of age were associated with early puberty.[28] The study added to the body of evidence that shows the importance of BMI monitoring from the beginning of life, to avoid early puberty and its implications.

We limited our subject recruitment to girls who had their first menstrual period within the 3 months prior to recruitment, with the aim of a short time period between menarche and data collection, so that the data would reflect measurements at menarche more accurately. Because we relied on subjects to recall the date of their first menstrual period, the maximum 3-month recall period would also help to reduce recall bias.

We measured 15 fat mass variables, but only two variables (BMI-for-age and waist-height ratio) had significant relationships with menarcheal age. This may have been due to the heterogenous nature of subjects' characteristics, which led to a broad range of body fat measurements. This limitation could have been mitigated by stratifying the study sample into early menarche, normal menarche, and late menarche groups. As such, a larger sample size would be required depending on the number of stratification groups. But the study results would be more representative of actual conditions.

To our knowledge, our study is the first in Indonesia to investigate the relationships between menarcheal age and body fat mass as well as fat distribution using BIA. The BIA was chosen for its accuracy, ability to assess fat distribution, ease of use, comfort during use, and relatively affordable cost. To further ensure accuracy, anthropometric measurements were done by the authors, taking a mean of two measurements, to reduce interobserver and intraobserver errors.

During puberty, teenagers undergo changes in body composition, especially body fat. Body fat assessment is ideally performed several times to monitor pre- and post-menarche changes. The limitations of this study were that the body fat mass and fat distribution measurement was done only once, and it was not done immediately after menarche. In order to increase accuracy, we limited the subjects we recruited to girls who had just experienced menarche and did not have ≥ 5% change in body weight in the last 3 months. The relationship between body fat and menarcheal age is influenced by leptin and other reproductive hormones (gonadotropin-releasing hormone, follicle-stimulating hormone, luteinizing hormone, and estradiol). In order to investigate the molecular processes of these relationships, a prospective cohort study with hormone monitoring through the pre- and post-menarche period would be the ideal design for future studies. In conclusion, body fat mass and fat distribution are not correlated with menarcheal age. However, there are weak to moderate negative correlations between menarcheal age and BMI-for-age as well as waist-height ratio.

Conflict of Interest

None declared.

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Original Article

Hepatitis A in children: evaluation of atypical manifestations

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Abstract

Background Although hepatitis A infection is known as a benign, self-limited disease without chronicity, the rate of complications increases over time.

Objective To evaluate atypical manifestations of hepatitis A infection in children.

Methods A total of 130 children with hepatitis A infection were reviewed. Subjects' demographic and clinical characteristics, laboratory examinations, and clinical courses were evaluated retrospectively.

Results Twenty-one subjects had atypical manifestations of disease as follows: immune thrombocytopenic purpura (1 patient), pleural effusion (1), autoimmune hepatitis and hemolytic anemia (1), nephrotic syndrome (2), meningoencephalitis (2), autoimmune hepatitis (2), acalculous cholecystitis (3), relapsing hepatitis (4), and fulminant hepatitis (5). Only gender was significantly different, with males having more atypical manifestations than females (P=0.03). Mortality rate was 3% (3 patients with fulminant hepatitis and 1 with meningoencephalitis died in the intensive care unit).

Conclusion Although hepatitis A virus infection has a benign, self-limited course without chronicity, recognition of atypical cases which carry mortality risk is important. **[Paediatr Indones. 2020;60:239-43 ; DOI: 10.14238/pi60.5.2020.239-43]**.

Keywords: *complication; hepatitis A; infection; liver failure*

Hepatitis A virus (HAV) is one of the most common infectious etiologies of acute hepatitis, which usually causes an asymptomatic infection in childhood. The symptoms and severity of infection vary according to age. Only 30% of children younger than 6 years of age with HAV infection develop symptoms which are typically non-specific, whereas 70% of infected adults have symptoms.[1,2] Although it is known as a benign, self-limited disease without chronicity, the rate of atypical manifestations and complications increases over time in patients with HAV infection.[3]

The prevalence of atypical manifestations such as relapsing hepatitis, cholestasis, autoimmune hepatitis, hematological abnormalities, pleural effusion, pancreatitis, acute renal failure, and fulminant hepatic failure (FHF) varies from 1 to 30% (overall mean of 7%).[2-6] There are a limited number of reports on pediatric HAV cases with atypical courses,[4-18] thus, the aim of this study was to evaluate atypical manifestations of HAV infection in pediatric patients and determine the course of the disease.

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Methods

A total of 130 children referred to the Division of Pediatric Gastroenterology, SBU Sisli Hamidiye Etfal Training and Research Hospital for HAV infection between 2003 and 2011 were evaluated retrospectively. Subjects underwent liver function tests [total bilirubin, direct bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST)], coagulation tests [prothrombin time (PT), activated partial thromboplastin time (aPTT), and international normalized ratio (INR)], as well as serological tests for hepatitis A, B, and C virus by enzyme-linked immunosorbent assay (ELISA).

Patients with histories and signs of pre-existing chronic liver disease were excluded from the study. None of the subjects had a history of hepatotoxic drug ingestion. Parents provided written informed consent before procedures were done.

Relapsing hepatitis A was defined as a period of remission characterized by resolution of symptoms and normalization of biochemical parameters lasting 4 to 5 weeks followed by a second episode with clinical and biochemical manifestations demonstrated by PCR viral replication of HAV in serum or feces.4 Fulminant hepatic failure was defined if the patient had (i) no known chronic liver disease from birth to 18 years; (ii) biochemical evidence of acute liver injury; and (iii) coagulopathy not corrected by parenteral administration of vitamin K [having either INR between 1.5 and 2.0 (PT ≥15 and <20 seconds) in the presence of hepatic encephalopathy (HE) or INR ≥2 (PT ≥20 seconds) with or without HE].[19,20]

Statistical analyses were performed using NCSS (Number Cruncher Statistical System) 2007 and PASS 2008 Statistical Software (Utah, U.S.A). Results are expressed as mean (SD). Statistical comparisons were made using unpaired Student's t-test and Chi-square test. A P value of <0.05 was considered to be statistically significant.

This retrospective study was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" (amended in October 2013).

Results

None of the subjects had received hepatitis A vaccines. Their demographic and clinical characteristics are shown in **Table 1**. The mean age of the 130 subjects was 6.1 (SD 4.1) years (range 1-16 years) and their male: female ratio was 1.03. Twenty-one of the 130 subjects [mean age: 6.47 (SD 3.2) years; M/F ratio 3.2] had atypical courses of disease (Table 2). Significantly more males than females had atypical courses of disease (P=0.016). Loss of consciousness, convulsion, nausea and vomiting, jaundice and hepatomegaly were significantly different between subjects with normal and atypical presentations (**Table 2**).

Table 1. Clinical and demographic characteristics of subjects

Characteristics	(N=130)
Mean age (SD), years	6.1 (4.1)
Male/female, n (ratio)	66/64 (1.03)
Symptoms, n (%)	
Nausea	26 (20)
Vomiting	87 (67)
Abdominal pain	56 (43)
Jaundice	83 (64)
Fever	26 (20)
Loss of appetite	52 (40)
Fatigue	52 (40)
Dark urine	32 (25)
Signs	
Hepatomegaly	84 (65)
Splenomegaly	3 (2.3)

The 21 subjects with atypical course of disease had the following conditions: immune thrombocytopenic purpura (ITP) (1 subject), pleural effusion (1), autoimmune hepatitis and hemolytic anemia (1), nephrotic syndrome (2), meningoencephalitis (2), autoimmune hepatitis (2), acalculous cholecystitis (3), relapsing hepatitis (4), and FHF (5). Two patients with relapsing hepatitis developed autoimmune hepatitis during their follow-up.

Conservative treatment was given to all subjects according to their diagnoses, such as corticosteroids for ITP and autoimmune hepatitis, anti-convulsants for meningoencephalitis, and treatment of hepatic coma in FHF. The mortality rate was 3% (3 FHF patients and 1 meningoencephalitis patient died in the intensive care unit).

Discussion

An increasing number of cases with atypical manifestations and complications of HAV infection in

Table 2. Clinical and demographic characteristics of subjects with atypical hepatitis A infection

Characteristics	Atypical hepatitis A infection (n=21)	Hepatitis A infection (n=109)	P value
Mean age (SD), years	6.47 (3.2)	6.2 (3.1)	0.71
Male/female ratio	16/5	50/59	0.016
Symptoms at admission, n			
Loss of consciousness	2	0	0.002
Convulsion	2	0	0.002
Nausea, vomiting	14	99	0.007
Jaundice	19	64	0.005
Cough	1	0	0.16
Abdominal pain	6	50	0.15
Clinical signs, n			
Hepatomegaly	7	77	0.002
Splenomegaly	2	1	0.06
Icterus	19	64	0.005
Petechiae	1	0	0.16
Laboratory findings of liver failure			
Mean ALT (SD), U/L (SD)	1.894 (1100)	1.978 (1336)	0.78
Mean AST (SD), U/L) (SD)	2.165 (1587)	1.677 (1094)	0.08
Mean total bilirubin (SD), mg/dL	7.5 (5.3)	7.54 (5.85)	0.97
Mean direct bilirubin (SD, mg/dL	4.5 (3.2)	5.52 (4.75)	0.34
Mean total protein (SD), mg/dL	6.9 (0.8)	6.3 (1.4)	0.059
Mean albumin (SD), mg/dL	3.5 (0.5)	4.1 (3.4)	0.42
Mean prothrombin time (SD), sec	14.5 (8.3)	15.6 (4.74)	0.39
Mean fibrinogen (SD), mg/dL	210 (75)	270 (160)	0.09

Normal lab parameters: ALT (10-35 U/L); AST (10-40 U/L); total bilirubin (0.3-1.2 mg/dL); direct bilirubin (<0.3 mg/dL); total protein (6.2-8 mg/dL); albumin (4-5.9 mg/dL); prothrombin time (10-14 sec); fibrinogen (200-400 mg/dL). P<0.05 is statistically significant.

children[4-18] and in adults[4,17,21-26] have been reported. A previous study observed that atypical manifestations were more common in HAV infection than in hepatitis B infection (30% *vs.* 3%, respectively; P=0.00).[6] The most common manifestation was prolonged cholestasis.[5] We observed that FHF (5/21) was the most common atypical manifestation followed by relapsing hepatitis (4/21). Another study reported that children with atypical presentations were older than the patients without atypical presentations.[5] Although we found no significant mean age difference between patients with atypical and normal presentations, we noted that significantly more males had atypical manifestations.

A study found that the rates of complications such as renal failure and cholestasis were significantly higher in adults >30 years of age than in children, but there was no significant difference in mortality according to age. Male patients predominated the group >30 years old in their study.[17] We observed significant male predominance in the group of patients with atypical manifestations.

A previous study has mentioned that most of the the studies including adults did not document relapse by PCR testing of viral HAV clearance, thus, it is difficult to establish the exact prevalence of relapsing hepatitis.[4] Another study reported the rate of relapsing hepatitis as 0.7%.[17] However, it was the second most common atypical manifestation of HAV infection in our study, with 4/21 of our atypical subjects experiencing it (3% of all subjects). The proposed pathophysiologic mechanism for relapsing hepatitis is that hav was not completely eliminated in the 1st episode so it replicated, leading to a 2nd episode in patients with reduced immunity who may not have produced adequate antibody titers to clear the virus from the body.[4] Our relapsing hepatitis patients were given conservative treatment and eventually complete clinical and biochemical resolution occurred.

A previous study observed that 38.7% of their patients had visible involvement of the gallbladder with >3 mm wall thickening, and 8% had acute acalculous cholecystitis. Thickening of the gallbladder wall was not significantly related to transaminase

levels, but was significantly associated with increased total and direct bilirubin levels.[16] A study noted the following significant associations between the presence of any ultrasonographic finding and peak total bilirubin levels, the presence of ascites with peak ALT and AST levels, and the presence of biliary sludge.[26] In our study, 3/21 of the atypical cases had acalculous cholecystitis and no significant associations were found between laboratory findings according to presence/absence of acalculous cholecystitis, possibly because of the small number of cases.

Acute liver failure due to HAV infection is more common in adults than children and in patients with underlying chronic liver diseas[e.4,27-29] The proposed pathophysiology is an exaggerated immune response, not the cytopathic effect of the virus in hepatocytes.[4] A study reported that the most common cause of FHF was hepatitis A infection in children aged less than 4 years. Such FHF patients had higher degree of encephalopathy and INR >4, and those with higher serum bilirubin and lower AST had poor outcomes.[15] Our FHF patients were also younger than 4 years.

The mortality rate was 3% in our study, mostly attributable to FHF as in the previous study.[7] Three out of 5 of our FHF patients (3/5) died during hospitalization in the intensive care unit without liver transplantation. Another study reported that 57.1% of their patients with FHF showed spontaneous survival, 37.1% received liver transplants, and 14.3% died.[3] Another study retrospectively reviewed 24 children with hepatitis A who showed evidence of liver failure in a single French urban pediatric liver transplantation center. They observed that encephalopathy occurred but resolved spontaneously in 7 children, and death or liver transplant were outcomes in 11 children (45.8%).[10]

The *Advisory Committee on Immunization Practices* (ACIP) has recommended hepatitis A vaccinations for all children during routine immunization, at the age of 12 to 23 months.[30] Preventive strategies targeting universal vaccination in children and active immunization of high-risk adults is important for prevention of community-wide outbreaks and complications of HAV infection.

In conclusion, primary health care providers should be aware of the potential atypical manifestations and complications of HAV infection and focus attention on childhood immunizations.

Conflict of Interest

None declared.

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Original Article

Diagnostic value of platelet indices for neonatal bacterial sepsis

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Abstract

Background Neonatal bacterial sepsis is a major cause of neonatal morbidity and mortality worldwide. Blood culture as a gold standard, as well as C reactive protein (CRP), micro erythrocyte sedimentation rate (micro ESR), white blood count (WBC), and immature-to-total (I/T) ratio as a sepsis screens are currently used methods, but their utility may be limited due to delayed reporting. Platelet indices are one of the parameters which can be helpful in the diagnosis of neonatal bacterial sepsis.

Objective To evaluate the use of platelet indices, either alone or in combination, with other laboratory screening parameters to *diagnose neonatal bacterial sepsis*.

Methods Neonates admitted to the Neonatal Unit of RSUP Dr. Muhammad Hoesin Hospital, Palembang, South Sumatera, and showing symptoms of sepsis were included in this study. Subjects underwent testing for blood culture, sepsis screen (CRP, micro ESR, WBC, I/T ratio), and platelet indices [platelet count, mean platelet volume (MPV), and platelet distribution width (PDW)].

Results The 107 neonates who fulfilled the inclusion criteria consisted of 42 neonates with proven bacterial sepsis (positive blood culture), 10 neonates with probable bacterial sepsis (positive sepsis screen and negative blood culture), and 55 with clinical bacterial sepsis (negative in both blood culture and sepsis screen). There were no significant differences in platelet count among the proven bacterial sepsis, probable bacterial sepsis, and clinical bacterial sepsis groups. Platelet count < 150,000/μL, PDW≥16.8 fL, MPV≥10.8 fL and combinations of the three, were highly specific markers for proven sepsis, with specificities of 92.3%, 97%, 75.4%, and 80%, respectively. However, all of these parameters were poor predictive markers for positive cultures in neonatal clinical bacterial sepsis, with sensitivities of 19%, 7.1%, 35.7%, and 23.8%, respectively.

Conclusion Platelet indices have high specificity but low sensitivity for the prediction of proven neonatal bacterial sepsis. **[Paediatr Indones. 2020;60:253-8 ; DOI: 10.14238/pi60.5.2020.253-8]**.

Keywords: *platelet indices; bacterial sepsis; neonatal sepsis*

Neonatal sepsis is a major cause of neonatal morbidity and mortality worldwide, contributing to around 38% of all deaths in neonates. The situation is even more in low income underdeveloped countries.[1] However, neonatal sepsis is a diagnostic challenge, as there are overlapping signs and symptoms which preclude a specific diagnosis of sepsis.[2] Neonates are fragile and can deteriorate rapidly, thus, early diagnosis and prompt treatment is required.[1,2] Clinical symptoms and signs of neonatal sepsis include the following: core temperature greater than 38.5°c or less than 36°c and/or temperature instability; cardiovascular instability: bradycardia in the absence of external vagal stimulus, beta-blockers or congenital heart disease or tachycardia in the absence of external stimulus, chronic drugs and painful stimuli and/or rhythm instability, reduced urinary output (less than 1 mL/kg/h), hypotension, mottled skin, impaired peripheral perfusion; skin and subcutaneous lesions:

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petechial rash, sclerema; respiratory instability: apnoea or tachypnea episodes; gastrointestinal disturbances: feeding intolerance, poor sucking, abdominal distention; irritability, lethargy, and hypotonia.[3] Laboratory signs of neonatal sepsis including: white blood cell (WBC) count: <5,000 cells/mm^3 or > 34,000 cells/mm^3;[4] immature-to-total neutrophil ratio (I/T) ≥0.2;[3,4] C-reactive protein (CRP) > 9 mg/dL;[5] micro-ESR > 15 mm/hour.[4]

There is no ideal test or combination of tests that serve as benchmarks for diagnosis. Blood culture has always been the gold standard for the diagnosis of neonatal sepsis, but its usefulness is limited due to low positivity rates, delayed reporting, and high cost. To overcome these limitations, we usually rely on sepsis screening (CRP, micro ESR, WBC, and IT ratio), but its sensitivity and specificity vary.[1,2]

Hematological changes induced by culture proven and probable neonatal sepsis have been used to make an early diagnosis and detect complications. Besides other hematological findings, changes in platelet count and platelet indices induced by neonatal sepsis have been the focus of many studies.[6] Thrombocytopenia is a non-specific indicator of neonatal sepsis, with or without disseminated intravascular coagulation (DIC). It can be caused by bacterial, viral, fungal, and parasitic infections, as well as other non-infectious conditions.[7] Advantages of platelet indices are that the blood specimen for these can be drawn at the same time as for other investigations, require no special sampling techniques, and are easily available.[8]

A previous study reported that platelet indices may be helpful in the diagnosis of neonatal bacterial sepsis.1 To our knowledge, there are not many studies on this topic from our region. Hence, we aimed to to evaluate the use of platelet indices, either alone or in combination with existing sepsis screens, as a marker of neonatal bacterial sepsis.

Methods

This cross-sectional study was conducted over a period of 8 months from February to September 2019 in the Neonatal Division, Department of Child Health, Muhammad Hoesin Hospital, Palembang, South Sumatera, to evaluate for relationships between neonatal bacterial sepsis and platelet indices (platelet count, MPV, and PDW). While the primary objective was to evaluate platelet indices (platelet count, PDW, MPV) as a marker of neonatal bacterial sepsis, the secondary objective was to determine differences in platelet indices among proven bacterial sepsis, probable bacterial sepsis, and clinical bacterial sepsis groups. There were 107 subjects who met the inclusion criteria of neonates aged 0-28 days with clinical symptoms or signs suggestive of neonatal sepsis, gestational age 24-42 weeks, and never received antibiotic treatment. Neonates with congenital and acquired causes of thrombocytopenia other than sepsis, i.e., autoimmune disorders of platelets, allo-immune disorder of platelets, maternal anti-platelet medication use, intrauterine growth retardation (IUGR), and incomplete data were excluded. Thrombocytopenia is defined as a platelet count < 150,000/µL. The normal value of MPV was < 10.8 fL and PDW was < 16.8 fL.[1,8]

Soon after admission, 3 mL blood specimens were taken and processed for blood culture, total leukocyte count (TLC), micro-ESR, I/T neutrophil ratio, and CRP. Platelet indices (platelet count, MPV, PDW) were also determined with an automated hematology analyzer (SYMEX XN-1000R). Blood culture was observed for 5-7 days before labelling it sterile. The culture and sensitivity report was done by Bactec method.[9] All the above tests were done in the microbiology laboratory. Subjects were categorized into three groups:

1. Proven sepsis: characterized by positive blood culture with clinical and/or laboratory evidence of sepsis.
2. Probable sepsis: blood culture negative, but meeting the criteria of presence of at least two clinical symptoms and at least two laboratory signs (sepsis screen).
3. Clinical sepsis: presence of at least two clinical symptoms, but with neither laboratory signs nor positive blood culture.

Univariate and bivariate analysis was done using *SPSS version 21* statistical software. Categorical data were shown in numbers and percentages. Numerical data were shown in mean (standard deviation) or median (minimum-maximum) according to the

normality of data distribution. Independent T-test and Mann-Whitney U test were performed to compare laboratory parameters between proven, probable, and clinical bacterial sepsis. Variables with significant results were analyzed further to determine sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy. The value of $P<0.05$ were considered statistically significant with 95% confidence interval.

Results

Characteristics of the 107 subjects are shown in **Table 1**. There were no significant differences in age, sex, type of delivery, and frequency of pregnancy between the bacterial sepsis (probable and/or proven) and clinical sepsis groups. However, lower gestational age and birth weight were significantly more dominant in the bacterial sepsis (probable and/or proven) than in clinical sepsis group ($P<0.05$).

Table 2 shows the most common signs and symptoms found in subjects. Of our 107 subjects, 42 (39.3%) were cases of proven bacterial sepsis (positive blood culture), 10 (9.3%) were categorized as probable sepsis, and 55 (51.4%) were categorized as clinical sepsis. The types of microorganisms cultured from those with proven sepsis are shown in **Table 3**. Platelet indices were compared between the proven and clinical bacterial sepsis groups in **Table 4**. Mann Whitney U statistical analysis revealed no significant differences in the number of platelets, MPV, and PDW between the proven and clinical bacterial sepsis groups, while **Table 5** shows the Mann Whitney U statistical analysis revealed no significant differences in the number of platelets, MPV, and PDW between groups.

The calculated diagnostic values of platelet indices for diagnosis of bacterial sepsis are shown in **Table 6**. The diagnostic values of probable bacterial sepsis that are commonly used (<5,000 cells/mm^3 or > 34,000 cells/mm^3, I/T ratio $\mu \geq 0.2$, CRP > 9 mg/dL, and micro-ESR > 15 mm/hour) are shown in **Table 7**.

Probable bacterial sepsis with ≥ 2 positive parameters had a 42.9% sensitivity, 84.6% specificity, 64.3% positive predictive value, 69.6% negative predictive value, and 68.2% accuracy (**Table 7**).

Table 1. Subjects' characteristics

Characteristics	Sepsis (proven and probable) (n= 52)	Clinical sepsis (n = 55)	P value
Age, n (%)			0.102[a]
<72 hours	17 (32.7)	26 (47.3)	
3 - 7 days	19 (36.5)	21 (38.2)	
> 7 days	16 (30.8)	8 (14.5)	
Gender, n (%)			0.627[b]
Male	28 (53.8)	26 (47.3)	
Female	24 (46.2)	29 (52.7)	
Gestational age, n (%)			0.000[b]
<37 weeks	36 (69.2)	17 (30.9)	
37-42 weeks	16 (30.8)	38 (69.1)	
>42 weeks	0 (0)	0 (0.0)	
Birth weight, n (%)			0.005[b]
<2,500 gr	30 (57.7)	16 (29.1)	
2,500-4,000 gr	22 (42.3)	39 (70.9)	
> 4,000 gr	0 (0)	0 (0)	
Type of delivery, n (%)			0.136[a]
Normal	32 (61.5)	39 (70.9)	
Caesarean section	19 (36.5)	12 (21.8)	
Forceps	1 (1.9)	4 (7.3)	
Pregnancy, n (%)			0.071[b]
Primigravida	43 (82.7)	36 (65.5)	
Multigravida	9 (17.3)	19 (34.5)	

a=Pearson Chi-square, P=0.05 ; b=continous correction, P=0.05

Table 2. Subjects' common signs and symptoms (N=107)

Signs and/or symptoms	n (%)
Poor suckling	68 (63.5)
Diarrhea/bloating/vomiting	44 (41.1)
Fever	40 (37.4)
Hypoactive/ irritable	34 (31.8)
Tachypnea	26 (24.3)
Seizure	8 (7.5)
Umbilical infection	5 (4.7)

Note: (1 subject had μ≥2 symptoms)

Table 3. Microorganisms cultured from 42 subjects

Microorganism	n(%)
Gram positive	16 (38.1)
Staphylococcus haemolyticus	9 (21.4)
Staphylococcus epidermidis	3 (7.1)
Staphylococcus aureus	2 (4.7)
Staphylococcus hominis Ssp hominis	1 (2.4)
Staphylococcus citreus	1 (2.4)
Streptococcus pyogenes	1 (2.4)
Streptococcus mitis/ oralis	
Gram negative	4 (9.5)
Klebsiella pneumoniae Ssp pneumoniae	2 (4.7)
Acinetobacter baumannii	2 (4.7)
Escherichia coli	1 (2.4)
Burkholderia cepacea	
Total	42 (100)

Table 4. Mean platelet indices in the proven and clinical bacterial sepsis groups

Parameters	Proven sepsis (n=42)	Clinical sepsis (n=55)	P value
Mean platelet count (SD), $10^3/\mu L$	305.5 (177.1)	310.6(132.3)	0.570
Mean MPV (SD), fL	10.63 (1.035)	10.27 (0.824)	0.057
Mean PDW (SD), fL	12.36 (3.342)	11.48 (2.138)	0.303

Table 5. Mean platelet indices in the probable and clinical bacterial sepsis groups

Parameters	Probable sepsis (n=10)	Clinical sepsis (n=55)	P value
Mean platelet count (SD), $10^3/\mu L$	299.4 (116.5)	310.6 (132.3)	0.985
Mean MPV (SD), fL	10.51 (1.124)	10.27 (0.824)	0.422
Mean PDW (SD), fL	12.35 (2.241	11.48 (2.138)	0.153

Discussion

In our study, we grouped probable and proven bacterial sepsis patients together (52/107; 48.5%); subjects with proven bacterial sepsis were 42/107 (39.2%). Hence, the sepsis group consisted of probable bacterial sepsis (10/52 subjects) and proven bacterial sepsis (42/52 subjects). The most common clinical signs and symptoms in our subjects were poor suckling, gastrointestinal disorders, temperature instability, hypoactivity, and respiratory disorders. A previous study noted that common clinical signs of studied groups were poor suckling (42%), lethargy (30%), poor Moro reflex (14%), and respiratory distress (8%).[10]

Blood cultures were positive in 42 subjects. The most commonly cultured microorganisms were Gram-positive bacteria in 33/42 (78.5%) subjects consisting of *Staphylococcus haemolyticus* (38%), *Staphylococcus epidermidis* (21.4%), *Staphylococcus aureus* (7.1%), *Staphylococcus hominis* (4.7%), and *Staphylococcus citreus*, *Streptococcus pyogenes*, and *Streptococcus mitis/ Streptococcus oralis* (2.3%). Gram negative bacteria were cultured from 9/42 (21.5%) subjects and consisted of *Klebsiella pneumoniae Ssp pneumoniae* (9.5%), *Acinetobacter baumannii* (4.7%), *E. coli* (4.7%), *Burkholderia cepacea* (2.3%). A previous study reported that of 469 patients, 136 (29%) were cases of culture proven sepsis, and 333 (71%) were categorized as probable sepsis. Among culture proven sepsis cases, 84 (61.8%) had Gram positive pathogens and 52 (38.2%) had Gram negative sepsis.[6] In contrast, another study reported that most cultured microorganisms were Gram negative (67.5%).[7]

In our study, the probable sepsis group had the lowest platelet count, while the proven sepsis group had the highest MPV and PDW. These findings indicate that patients with low platelet levels, high MPV, or high PDW become septic. In contrast, a previous study showed a significant difference in platelet indices of their sepsis group compared to the control group (healthy neonates).11 Our study showed that platelet count < 150,000/μL, PDW ≥16.8 fL, and MPV ≥10.8 fL individually and in combination were highly specific markers for predicting proven bacterial sepsis, with specificities of 92.3%, 97%, 75.4%, and 80%, respectively. However, these parameters were poor predictive

Table 6. Performance variables of platelet indices for diagnosis of bacterial sepsis compared to blood culture as the gold standard in proven sepsis group (n=42)

Diagnostic value	Platelet <150x10^3/µL	MPV ≥0.8 fL	PDW ≥16.8 fL	Combination ≥2 parameter
Sensitivity, %	19	35.7	7.1	23.8
Specificity, %	92.3	75.4	97	80
NPV, %	63.8	64.5	64.5	62
PPV, %	61.5	48.4	60	43.5
Accuracy, %	63.5	55.1	61.7	57.9

Note: combination ≥ 2 paramaters consisted of Plt + MPV or Plt + PDW, or MPV + PDW

Table 7. Performance variables of sepsis screen for diagnosis of probable bacterial sepsis compared to blood culture results

Parameters biomarker sepsis screen		Blood culture		Total
		Positive	Negative	
WBC > 34x10^3/mm^3 or < 5x10^3/mm^3	≥ 2 positive parameters	18	10	28
I/T ratio ≥0.2 ESR > 15 mm/hour CRP > 9 mg/dL	< 2 positive parameters	24	55	79
Total		42	65	107

markers for culture positivity in clinical bacterial sepsis, with sensitivities of 19%, 7.1%, 35.7%, and 23.8%, respectively. We noted that ≥ 2 positive sepsis screen parameters had higher accuracy (68.2%) and higher Youden index score than platelet indices. The accuracy of 68.2% indicates that the degree of measurement conformity (reliability) was good.

A previous study found that platelet count was the most sensitive marker for sepsis, with 83.70% sensitivity, followed by 75.20% for MPV and 66.70% for PDW. Their specificity of platelet count was also highest at 65%, followed by 64.30% for MPV and 57.80% for PDW. When any two of the platelet indices were combined, the specificity increased to a maximum of 67.0% (platelet count and MPV combined). The maximum sensitivity was 85.80% (platelet count and MPV combined) as a marker for sepsis. However, when all three parameters were taken together, the sensitivity was 84.10% and specificity was 65.50%.1 Likewise, another study in Egypt reported MPV sensitivity and specificity values of 100% each, in their study of 140 subjects with healthy individuals as a control group.[10]

In conclusion, platelet indices have high specificity but low sensitivity for the prediction of proven neonatal bacterial sepsis.

Conflict of Interest

None declared.

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Original Article

Investigating *Minimum Acceptable Diet* and *Infant and Child Feeding Index* as indicators of stunting in children aged 6-23 months

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Abstract

Background The complementary feeding period of 6-24 months of age is one of the most crucial moments in child growth, in which most of the decline in length-for-age Z-score (LAZ) occurs. The *Minimum Acceptable Diet* (MAD) and *Infant and Child Feeding Index* (ICFI) are indicators to assess complementary feeding practices in the children with potential for stunting.

Objective To assess and compare the usefulness of MAD and ICFI scores as indicators of inadequate feeding practice on stunting in children aged 6-23 months.

Methods This case-control study was conducted in South and West Wewewa subdisctricts of Southwest Sumba, East Nusa Tenggara, Indonesia, from February to August 2019. Participants were children aged 6-23 months who had received complementary feeding for a minimum duration of one month. Children with LAZ <-2 were allocated into the case group (stunted) and those with LAZ>-2 into the control group. Both MAD and ICFI scores were assessed in both groups. ICFI was categorized as low, average, and high. The association between complementary feeding practice which depicted by the MAD and ICFI scores and stunting was measured using logistic regression.

Results Of 322 participants, 161 children were allocated into each group. Multivariate analysis revealed that those in low and average ICFI tertile had higher odds of stunting [(OR 2.85; 95%CI 1.35 to 6.00; P<0.01) and (OR 1.95; 95%CI 1.09 to 3.46; P<0.05), respectively]. No association was found between MAD and stunting.

Conclusion Inadequate complementary feeding practice is found to increase the risk of stunting among children aged 6-23 months. Compared to MAD, ICFI is a better indicator in demonstrating an association between complementary feeding practice and stunting. **[Paediatr Indones. 2020;60:259-68 ; DOI: 10.14238/pi60.5.2020.259-68]**.

Keywords: *stunting; complementary feeding; infant and child feeding index; minimum acceptable diet*

Around 30.8% of Indonesian children under five years of age were classified as stunted and severely-stunted.[1] The province of East Nusa Tenggara had the highest stunting prevalence since 2007, and has not made significant progress towards stunting reduction as of 2018.[1,2] Around 42.6% of under-fives and 29.8% of under-twos in the province were stunted.[1,3] Southwest Sumba is one of the municipalities in the province prioritized for the 2017 National Stunting Reduction Program due to its high number of stunted children totaling 26,809 under-fives (61.2%).[4]

The period between birth and two years of age has been identified as the most vulnerable period for becoming undernourished, due to the need for a high quality diet to support optimal growth and development.[5] Most of the decline in length-for-age Z-score (LAZ) occurs during the complementary feeding period between 6-24 months of age. Poor feeding practices with insufficient quantity and

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inadequate quality of complementary foods are important direct risk factors of stunting.[6,7]

Inadequate infant and young child feeding (IYCF) practice is still a common problem in developing countries.[8,9] Inadequate feeding, in terms of poor macronutrient and micronutrient quality, is caused by poor dietary diversity as well as inappropriate energy and nutritional density. Inappropriate feeding frequency and food quantity also contribute to inadequate energy and nutritional intake.[8] Complementary feeding practices vary and are influenced by many factors, thus standard indicators to assess these practices are needed to determine the complementary feeding situation in a region.[7]

The World Health Organization (WHO) developed complementary feeding indicators to assess feeding practices in children between age 6-23 months. The core indicators include *Minimum Dietary Diversity* (MDD), *Minimum Meal Mrequency* (MMF), and *Minimum Acceptable Diet* (MAD).[7,10] The MAD has been used globally as one of the main indicators to assess the adequacy of feeding practices.[7,10,11] Unachieved MAD was associated with stunting in children aged 6-23 months.[10,12] Another indicator to assess IYCF practice is the Infant and Child Feeding Index (ICFI).[13] Its development was based on data from five Latin American countries and includes five components of complementary feeding practice: breastfeeding, bottle-feeding, dietary diversity, food frequency in the previous seven days, and meal frequency.[5,10,13,14] The ICFI score was associated with LAZ in children under five years of age.[8]

Considering the importance of appropriate feeding practices on growth and stunting prevention in children aged 6-23 months, we aimed to assess feeding practices in the high stunting prevalence area of Southwest Sumba. To our knowledge, no such study has been conducted in this area. Thus, we used unachieved MAD and low ICFI scores to assess for inadequate feeding practice in stunted children aged 6-23 months in the Wewewa subdisctrict, Southwest Sumba.

Methods

This community-based, unmatched case-control study was conducted in the South and West Wewewa subdisctricts, Southwest Sumba, East Nusa Tenggara, from February to August 2019 to assess and compare MAD and ICFI scores as indicators of inadequate feeding practice in stunted children aged 6-23 months. Participants were children aged 6-23 months who had received complementary feeding for a minimum duration of one month at the time of data collection and who visited the nutrition clinic and integrated health service posts (Posyandu) of the Tena Teke Community Health Center (CHC) with their biological mothers. The Tena Teke CHC serves 16 villages situated in South and West Wewewa with an estimated total population of 43,333 people.[15] Children with LAZ <-2 or "stunted" based on the 2006 WHO child growth standard were allocated into the case group and those with LAZ ≥-2 were allocated to the control group.[16] Mothers provided written informed consent before each interview session. Children with previously diagnosed or treated nutritional disorders, acute conditions affecting oral intake, chronic conditions, congenital anomalies, or preterm, post-term or multiple birth history were excluded.

The minimum required sample size was calculated for an unmatched case-control study. Based on a previous study, the proportion of unachieved MAD in children aged 6-23 months with normal LAZ (P2) was 58%.[9] If the predetermined significant odds ratio was 2.0 with 95%CI ($Z\alpha=1.96$), power of 80% ($Z\beta=0.842$), and sample size ratio of 1:1 for control and case groups, the minimum required sample size was estimated to be 158 for each group. Participants were chosen using a convenience sampling method until the minimum required sample size for each group was fulfilled.

Recumbent body length was measured using a *Kenko®* infantometer with 0.1 cm precision. Body length was measured twice and the mean of the two measurements was used for the final analysis. The LAZ was calculated using the *Emergency Nutrition Assessment (ENA) for SMART 2011* (*SMART Org.*, Toronto, Canada). Demographic characteristics and complementary feeding practices of the participants were collected using a pre-validated questionnaire. Dietary diversity and meal frequency were assessed using a 24-hour food recall technique with the help of a food atlas. Prior to general use by all subjects, the questionnaire was validated by a pediatrician for its

content and was pre-tested on 5% of the participants to evaluate the language clarity and consistency.

The child's gender, maternal education and work status, birth order, family size, parental income, sanitation, and episodes of fever, diarrhea, and upper respiratory tract infection in the past two weeks were compared between the case and control groups. Participants' age was presented in mean values and categorized into three age groups to depict the difference of age distribution between case and control groups. Parental income was categorized as "sufficient" if the value was ≥ IDR 1 million/month and "not sufficient" if otherwise. For sanitation, which defined by the WHO as access to and use of facilities and services for the safe disposal of human urine and feces, "improved" and "not improved" status were used.[17] An "improved" sanitation status was given if the child live in a household that had a safe excreta disposal system which was a toilet with septic tank or pit latrine with or without slab and the household had its own toilet or latrine. If one of these conditions were not met, the child was labeled as having "not improved" sanitation.[17]

The main indicators of complementary feeding practice were MAD and ICFI scores. The MAD was defined by the WHO as at least meeting the *Minimum Dietary Diversity* (MDD) and *Minimum Meal Frequency* (MMF) during the previous day. The MDD was defined as receiving foods from ≥4 food groups, including (1) grains, roots and tubers; (2) legumes and nuts; (3) dairy products (milk, yogurt, cheese); (4) flesh foods (meat, fish, poultry and liver/organ meats); (5) eggs; (6) vitamin-A rich fruits and vegetables; and (7) other fruits and vegetables. The MMF was defined as receiving solid, semi-solid, or soft foods including milk feeds for non-breastfed children for the minimum number of times or more during the previous day. For breastfed children, the minimum was defined as 2 times daily for infants aged 6-8 months and 3 times daily for children aged 9-23 months. For non-breastfed children, the minimum was defined as 4 times daily for children aged 6-23 months.[18]

The ICFI, as previously mentioned, consisted of five different components as shown in **Table 1**. The ICFI assessment was divided into 3 age categories, each with a total maximum score of 12.[13] The dietary diversity score assessed the consumption of complementary food from food groups using a 24-hour food recall technique. Meal frequency assessed the intake of solid, semi-solid, and soft foods in the previous 24 hours. In addition, the food frequency score component reflected the consumption of certain food groups in the previous 7 days. Total ICFI scores were categorized into tertiles as low (ICFI score of ≤4), average (ICFI score of 5-6), and high (ICFI score of ≥7).[13,19] Each of the ICFI components and the mean total ICFI score were also compared between case and control groups.

Characteristics of participants and the achievement of ICFI indicators were presented as proportions and percentages. Mean age and total ICFI score were presented as continuous data. Bivariate analyses of the categorical data were done using Pearson's Chi-square and Fisher's exact tests and analysis of continuous data was done using Mann-Whitney U test. Logistic regression analysis was used to compare MDD, MMF, MAD, and ICFI tertiles with stunting. Sociodemographic characteristics that had a P values of <0.25 were included in the final logistic regression model for adjustment. Odds ratio (OR) with 95%CI was presented as the final result. Results with P values <0.05 were considered to be statistically significant. Statistical analyses were done using *Statistical Package for Social Science (SPSS) version 21.0* (*SPSS Inc.*, Chicago, IL, USA). This study was approved by the National and Political Unity Unit (Kesbangpol) of the Southwest Sumba municipality.

Results

A total of 322 children aged 6-23 months and their mothers were enrolled, with 161 children allocated into each group. Sociodemographic characteristics of the case and control groups are presented in **Table 2**. Around 56% of the children were male and there was no significant gender difference between groups. In contrast, children in the case group were significantly older than those in the control group, with mean ages of 14.9 (SD 4.9) and 11.2 (SD 4.3) months, respectively (P<0.001). Also, 69.6% of children in the case group were 12-23 months of age, compared to only 32.3% of the control group.

Lower maternal educational level and unemployment were significantly higher in the case than the control group. About half of the mothers

Table 1. The ICFI components based on age groups[13]

Variables	6-9 months	9-12 months	12-36 months
Breastfeeding	No = 0 Yes = 2	No = 0 Yes = 2	No = 0 Yes = 1
Use of bottle	No = 0 Yes = 2	No = 0 Yes = 2	No = 0 Yes = 2
Dietary diversity (previous 24 hours)	Sum of: (grains + tubers + milk + egg + meat/fish/poultry + vitamin A-rich fruits and vegetables + other fruits/veg) 0=0 1-3=1 ≥4=2	Sum of: (grains + tubers + milk + egg + meat/fish/poultry + vitamin A-rich fruits and vegetables + other fruits/veg) 0=0 1-3=1 ≥4=2	Sum of: (grains + tubers + milk + egg + meat/fish/poultry + vitamin A-rich fruits and vegetables + other fruits/veg) 0=0 1-3=1 ≥4=2
Food frequency (previous 7 days)	For each of: • egg/fish/poultry • meat 0 times in previous 7d=0 1-3 times in previous 7d=1 ≥4 times in previous 7d=2 For staples (grains or tubers) 0-2 times=0 ≥3 times=1 Food frequency=sum of scores for staples + egg/fish/poultry + meat	For each of: • egg/fish/poultry • meat 0 times in previous 7d=0 1-3 times in previous 7d=1 ≥4 times in previous 7d=2 For staples (grains or tubers) 0–3 times=0 ≥4 times=1 Food frequency=sum of scores for staples + egg/fish/poultry + meat	For each of: • milk • egg/fish/poultry • meat 0 times in previous 7d=0 1-3 times in previous 7d=1 ≥4 times in previous 7d=2 Food frequency=sum of scores for milk + egg/fish/poultry + meat
Meal frequency* (previous 24 hours)	0 meals/day=0 1 meal/day=1 2 meals/day=2	0 meals/day=0 1-2 meals/day=1 ≥3 meals/day=2	0-1 meal/day=0 2-3 meals/day=1 ≥4 meals/day=2
Maximum total score	12 points	12 points	12 points

Note: meal frequency did not include breastmilk or other liquids and only refered to solid, semi-solid, or soft foods received in the previous 24 hours

in the case group had less than secondary school education and around two-thirds were unemployed. Birth order, family size, family income, sanitation level, and episodes of fever, diarrhea and upper respiratory tract infection (URTI) were not significantly different between groups.

With regards to the WHO IYCF indicators, more than 80% of all subjects did not achieve MAD and MDD (**Table 3**). The MMF was slightly better, with achievement by more than half of all subjects. Bivariate analysis revealed that MAD, the main WHO IYCF indicator, was not associated with stunting. This result was similar for MDD. The MMF was the only indicator associated with stunting (P=0.001). Significantly more unachieved MMF was found in children in the case group compared to the control group (40.4% *vs.* 23.6%, respectively; P<0.01).

For ICFI tertiles, only approximately one-fourth of the case group achieved high ICFI score compared to more than half of the control group (**Table 3**). Also, significantly more children in the case group had low ICFI score compared to the control group (36.0% *vs.* 12.4%, respectively). Bivariate analysis showed that significantly more stunted children had low ICFI scores than non-stunted controls. The mean total ICFI score of the case group was 5.3 (SD 1.6), which was significantly lower than that of the control group [6.4 (SD 1.4)]. After stratifying ICFI scores by age category, we found that total ICFI scores were the highest in the 6 to 8-month age group, and decreased in the older age groups, as shown in **Table 4**. In the 12 to 23-month age group, mean total ICFI was significantly lower in the case group compared to the control group [4.6 (SD 1.4) *vs.* 5.2

Table 2. Sociodemographic characteristics of subjects

Characteristics	Case (n=161)	Control (n=161)	P value
Gender, n(%)			
Male	97 (60.2)	86 (53.4)	0.216
Female	64 (39.8)	75 (46.6)	
Age group, n(%)			
6-8 months	23 (14.3)	69 (42.9)	<0.001
9-11 months	26 (16.1)	40 (24.8)	
12-23 months	112 (69.6)	52 (32.3)	
Maternal education, n(%)			
< middle school	82 (50.9)	62 (38.5)	0.033
≥ middle school	79 (49.1)	99 (61.5)	
Maternal employment status, n(%)			
Working	49 (30.4)	70 (43.5)	0.015
Not working	112 (69.6)	91 (56.5)	
Birth order, n(%)			
≤2	80 (49.7)	83 (51.6)	0.738
>2	81 (50.3)	78 (48.4)	
Family size, n(%)			
≤4	81 (50.3)	85 (52.8)	0.656
≥5	80 (49.7)	76 (47.2)	
Family income, n(%)			
Insufficient	104 (64.6)	88 (54.7)	0.069
Sufficient	57 (35.4)	73 (45.6)	
Fever, n(%)			
Yes	101 (62.7)	101 (62.7)	1.000
No	60 (32.3)	60 (32.3)	
Diarrhea, n(%)			
Yes	19 (11.8)	26 (16.1)	0.261
No	142 (88.2)	135 (83.9)	
Upper respiratory tract infection, n(%)			
Yes	122 (75.8)	134 (83.2)	0.098
No	39 (24.2)	27 (16.8)	
Sanitation, n(%)			
Improved	109 (67.7)	117 (72.7)	0.33
Not improved	52 (32.3)	44 (27.3)	

Table 3. Bivariate analysis of complementary feeding practice indicators and stunting in the case and control groups

Indicators	Achieved	Case	Control	P value
Minimum Meal Frequency, n (%)	Yes	96 (59.6)	123 (76.4)	0.001*
	No	65 (40.4)	38 (23.6)	
Minimum Dietary Diversity, n (%)	Yes	24 (14.9)	26 (16.1)	0.758
	No	137 (85.1)	135 (83.9)	
Minimum Adequate Diet, n (%)	Yes	18 (11.2)	22 (13.7)	0.499
	No	143 (88.8)	139 (86.3)	
ICFI categories, n (%)	Low	58 (36.0)	20 (12.4)	<0.001**
	Average	65 (40.4)	52 (32.3)	
	High	38 (23.6)	89 (55.3)	

(SD 1.5), respectively; P<0.05]. But mean total ICFI were not significantly different in the younger age categories between the case and control groups.

As shown in **Table 5**, for all ages combined, the case group had significantly lower percentages than the control group with regards to breastfeeding (50.9% *vs.* 78.3%, respectively; P<0.001) and high meal frequency score (45.5% *vs.* 67%; respectively; P<0.001). In addition, bottle-feeding was significantly higher in the case group, with almost half of the children. There were no significant differences for dietary diversity score and food group frequency components between groups.

In contrast, when the components were stratified by age category, there were no significant differences between groups for all five ICFI components. Breastfeeding and meal frequency scores were highest in 6 to 8-month age category in both groups, and decreased in the older age groups.

After adjustment for sex, age, maternal education and work, family income, and URTI variables in the logistic regression model, low and average ICFI for all children was the only indicator with significant risks for stunting, as shown in **Table 6**. Compared to children in the high tertile, those in the low and average tertiles were more likely to become stunted [OR 2.85; (95%CI 1.35 to 6.00); P<0.01 and OR 1.95; (95%CI 1.09 to 3.46); P<0.05, respectively]. In contrast, no associations were found between the WHO IYCF indicators and stunting in the multivariate analysis.

Discussion

Our study confirms an association between inadequate complementary feeding practice and stunting among children 6-23 months in the Wewewa subdistrict, Southwest Sumba. ICFI as a composite index for assessing complementary feeding practice was found to reflect the risk of stunting. Furthermore, children in the low and average ICFI tertiles had higher odds

Table 4. Mean total ICFI scores based on age category

Age categories	Case		Control		P value
	n	Mean (SD)	n	Mean (SD)	
6-8 months	23	6.7 (0.9)	69	7.0 (0.9)	0.275
9-11 months	26	6.6 (1.2)	40	6.7 (1.2)	0.700
12-23 months	112	4.6 (1.4)	52	5.2 (1.5)	0.025
All age categories	161	5.3 (1.6)	161	6.4 (1.4)	<0.001

Table 5. Comparison of ICFI components by age category

ICFI component	6-8 months			9-11 months			2-23 months			All ages		
	Case n=23	Control n=69	P value[a]	Case n=26	Control n=40	P value[a]	Case n=112	Control n=52	P value[a]	Case n=161	Control n=161	P value[a]
Breastfeeding (%)												
Yes	95.7	95.7	1.00[b]	88.5	87.5	1.00[b]	33.0	48.1	0.06	50.9	78.3	<0.001
Bottle feeding (%)												
No	56.5	76.8	0.06	57.7	62.5	0.69	54.5	69.2	0.07	55.3	70.8	0.04
Dietary diversity score (%)												
Medium (1)	100	89.9	0.18[b]	84.6	87.5	0.73[b]	82.1	73.1	0.18	85.1	83.9	0.75
High (2)	0	10.1		15.4	12.5		17.9	26.9		14.9	16.1	
Food group frequency												
Low (0-2)	95.7	95.7	1.00[b]	92.3	95.0	0.64[b]	86.6	84.6	0.70	88.8	91.9	0.44
Medium (3,4)	4.3	4.3		7.7	5.0		12.5	15.4		10.6	8.1	
High (5,6)	0	0		0	0		0.9	0		0.6	0	
Meal frequency score												
Low (0)	0	0	0.33[b]	0	0	0.53	0.9	5.8	0.15	0.6	1.9	<0.001
Medium (1)	0	8.7		34.6	27.5		69.6	63.5		54.0	31.1	
High (2)	100	91.3		65.4	72.5		29.5	30.8		45.4	67.0	

a=Chi-square; b=Fisher's exact test

Table 6. Multivariate analysis of IYCF practice indicators and stunting after adjustment of several potential confounding variables

Indicators		OR[a]	95% CI	P value
MMF	Yes	1.00 (ref.)		
	No	0.83	0.44 to 1.54	0.55
MDD	Yes	1.00 (ref.)		
	No	0.95	0.43 to 2.07	0.90
MAD	Yes	1.00 (ref.)		
	No	1.28	0.23 to 7.14	0.77
ICFI category	Low	2.85	1.35 to 6.00	0.006
	Average	1.95	1.09 to 3.46	0.02
	High	1.00 (ref.)		

OR[a]=adjusted for sex, age, maternal education, maternal work, family income, and upper respiratory tract infection event

of being stunted compared to those in the high tertile, even after the adjustment of several potential confounding variables. The odds of stunting increased as the ICFI tertile decreased. Children in low ICFI tertile were 2.85 (95%CI 1.35 to 6.00; P<0.01) times more likely to be stunted compared to those in the high tertile. The odds were still nearly doubled even for children in the average tertile (OR 1.95; 95%CI 1.09 to 3.46; P<0.05) compared to those in the high tertile, indicating that better complementary feeding practice would reduce the risk of stunting.

In contrast, we found no association between MAD, as the main WHO IYCF indicator, and stunting. Nor were MDD or MMF associated with stunting. In addition, the majority of the children in our study were not adequately fed. Only less than 15% of children in both groups achieved MAD, and most subjects did not receive appropriate meal frequency or dietary diversity.

We did not find an association between the WHO IYCF main indicators (MMF, MDD, and MAD) and stunting. An Aceh, Indonesia study also confirmed this finding and showed no association between these indicators and stunting in children aged 6-23 months.[8] A study in Northern Ghana also showed no association.[9] A previous study concluded that WHO IYCF indicators might be better in explaining weight-for-height Z-scores (WHZ) than LAZ, and were more associated with acute malnutrition. The lack of association might have been caused by the low sensitivity of these indicators, since they were assessed based on a 24-hour reference period which best reflects recent diet.[9] However, a Nigerian study in children aged 6-11 months showed that infants who did not achieve the MDD and MMF were significantly more likely to be stunted [(OR 2.17; 95%CI 1.43 to 4.20; P<0.05) *vs.* (OR 1.57; 95%CI 1.53 to 4.03; P<0.05), respectively].[20] Considering this discrepancy, further studies are needed to address the association between WHO IYCF indicators and stunting in different regions of Indonesia.

The lack of association between WHO IYCF indicators and stunting indicates that ICFI would be a better tool to assess complementary feeding practices in children aged 6-23 months in relation to stunting. A Cambodian study similarly showed a significant association between LAZ and ICFI score, but no association between LAZ and WHO IYCF indicators.[10] ICFI was regarded as a more useful indicator in demonstrating an association between IYCF practices and LAZ, compared to the simpler WHO IYCF indicators. They also found that the more ICFI criteria met by caregivers, the more likely the children (aged 6-23 months) achieved age-appropriate length.[10]

Several studies have also demonstrated an association between complementary feeding practice which assessed by using the ICFI and linear growth of children aged 6-23 months.[5,10,19,21] A study from Burkina Faso, West Africa, showed that mean LAZ was significantly and positively related to ICFI tertiles in children aged 6-23 months.[19] In addition, a study in urban slums of Mumbai also confirmed this finding and showed a significant association between ICFI and LAZ. The ICFI was considered to better reflect chronic malnutrition in young children and

better capture representative information on various components of young child feeding practices.[5]

In our study, stunted children tended to receive poorer feeding practices compared to the normal controls. This finding was reflected by the lower mean total ICFI score in the case group compared to the control group. This result was in agreement with a rural Cambodian study showing that stunted children had significantly lower ICFI scores compared to those without stunting [6.4 (SD 1.5) *vs.* 6.8 (SD 1.8), respectively; (P=0.003)].[10]

After our groups were stratified by age category, mean total ICFI score was significantly lower in children aged 12-23 months. A previous study demonstrated the role of age as a moderator in the relationship between LAZ and ICFI. But interestingly, they found that the association was present only in children under 1 year of age, which differed from our results.[10] However, another study found that ICFI categories were positively related to mean LAZ in children aged 6-11 months and 12-23 months.[19] Thus, these results indicate that children in certain age categories might be more vulnerable to stunting due to inadequate IYCF practices in different settings.

Other studies have also demonstrated that some ICFI components might affect the risk of stunting in children aged 6-23 months more than other components.[10,19] We found that the breastfeeding, bottle-feeding, and meal frequency components of ICFI were significantly different between the case and control groups, although after age stratification, none of these components showed significant results. In addition, there were no significant differences in terms of dietary diversity and food group frequency between groups. A study also showed that LAZ scores were not associated with dietary diversity, but were positively correlated with higher food frequency.[10] In contrast, another study found that dietary diversity and feeding frequency were associated with LAZ.[19] They also concluded that dietary diversity was an important component of ICFI and that it mattered more than consumption of a particular food group. In addition, they noted that breastfeeding was negatively associated with LAZ, especially in children over 12 months of age.[19] Differing results from these studies indicate that some ICFI components might play more or less of a role than others in affecting child growth in certain settings. Thus, region-to-region assessment could be done to address which IYCF practice components have a predominant role in stunting in each region.

Appropriate instruments that accurately assess IYCF practices are crucial for successful interventions to improve child nutrition. However, consistent and reliable indicators to assess these practices remain limited. Since feeding practice is a multidimensional and age-specific process, a more comprehensive indicator is needed to fully capture feeding practices. The use of a composite index such as ICFI would combine various feeding dimensions that could quantify feeding practices into one objective variable.[22]

Although further studies are needed to test the applicability of ICFI in assessing IYCF practice in different Indonesian settings, our results suggest that ICFI as a composite index would be useful as an objective indicator to evaluate both IYCF practice and the improvement of feeding practices after intervention. However, interventions to improve complementary feeding practices should be made on a region-to-region basis because different components of feeding practices may have a more prominent role than others in affecting the risk of stunting in different settings.

Our study had several limitations. Since the development of stunting is a long-term process and feeding practices can vary on a day-to-day basis, the one point of data collection used in this study might not completely reflect feeding practices received by children or their impact on growth. In addition, since we used a non-randomized sampling method, generalizability of the results might be limited. Interviews on past events and the 24-hour food recall technique used in this study are also prone to recall bias, which might have affected the validity of the result. Considering these limitations, a longitudinal study with a randomized sampling method that periodically assesses IYCF practices and children's growth is needed to better capture the longitudinal relationship between complementary feeding practice and stunting. Despite these limitations, our study is the first to assess the impact of complementary feeding practice as reflected by the achievement of WHO IYCF indicators and ICFI score on stunting in the Southwest Sumba region, as a high stunting burden area in Indonesia. Also, to our knowledge, this is the first study in Indonesia to use ICFI as an indicator to

assess IYCF practices received by children aged 6-23 months.

In conclusion, inadequate complementary feeding practice is associated with the risk of stunting among children aged 6-23 months in the Wewewa subdistrict of Southwest Sumba, Indonesia. The odds of stunting are doubled in children who received poor complementary feeding practice. Compared to WHO IYCF indicators, ICFI, as one of the tools to assess IYCF practices, is found to better demonstrate the association between complementary feeding practice and stunting. Measures to improve complementary feeding practices among children aged 6-23 months may decrease the burden of stunting in Southwest Sumba. Further studies are needed to assess the validity of ICFI as a tool to evaluate the association between IYCF practices and stunting in different settings in Indonesia.

Conflict of interest

None declared.

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Case Report

Methylprednisolone as an alternative therapy for Kawasaki disease: case series

Yudha Fadhol Arafah, Sasmito Nugroho, Noormanto, Nadya Arafuri, Indah Kartika Murni

Kawasaki disease (KD), or mucocutaneous syndrome, is an acute, systemic vasculitis of small- and medium-sized arteries that predominantly affects patients younger than five years.[1] KD is the leading cause of childhood acquired heart disease in the developed world.[2] The incidence in those aged under 5 years varies widely throughout the world, accounting for 8.4 per 100,000 in the UK, 17.5 to 20.8 per 100,000 in the USA, and 239.6 per 100,000 in Japan.[2]

The diagnosis of classic KD is based on the simultaneous presence of high fever for 5 or more days with at least four of five other symptoms (bilateral conjunctival hyperemia, ulcerations of the lips and inflammation of the oral cavity, polymorphous rash, edema and desquamation of the extremities, and cervical lymphadenopathy), or fever associated with less than 4 of the diagnostic criteria and echocardiographic abnormalities of the coronary arteries.[3]

In the acute phase, KD treatment aims at reducing the inflammation in the coronary artery wall and preventing coronary thrombosis, whereas long-term therapy, especially in patients with coronary artery ectasias or aneurysms, aims at preventing myocardial damage. Approximately 10% to 20% of patients have persistent fever after standard therapy with intravenous immunoglobulin (IVIG) and oral acetylsalicylic acid, increasing their risk for the development of coronary artery lesions (CAL).[4] The main difficulties for clinicians are performing a timely diagnosis, preventing cardiovascular complications, and treating refractory forms. The incidence of refractory forms has markedly increased, with young age and delayed treatment as major risk factors.[3] In developed countries, IVIG for Kawasaki disease therapy may not be available in every province or rural areas. As such, it is important to have an alternative therapy in such cases. Corticosteroids were used to treat KD long before the first report of IVIG therapy.[5] Here we describe the clinical courses and outcomes of two children with KD who received methylprednisolone therapy. **[Paediatr Indones. 2020;60:283-6 ; DOI: 10.14238/pi60.5.2020.283-6]**.

Keywords: *platelet indices; bacterial sepsis; neonatal sepsis*

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The Cases

The first case was a 16-month-old boy presenting with five days of high fever who was referred from a private hospital in Cilacap to Banyumas District Hospital, Central Java. During the second day of fever, the patient had diarrhea and vomiting more than three times a day. On the fourth day of fever he had generalized erythema on both hands and feet, which spread over the body. On the sixth day of fever, the patient had red lips, strawberry tongue (**Figure 1**), conjunctival injection (**Figure 2**), polymorphous skin rash all over the body , and desquamation of the perineal area (**Figure 3**). On laboratory investigation, leukocyte level was 12,080/μL, with 84.6% neutrophils, hemoglobin 10.9 g/dL, and platelets 345,000/μL. His albumin level was 2.2 g/dL, sodium was 131 mEq/L, and CRP was 300 mg/L. Echocardiography showed minimal pericardial effusion. The patient was treated with methylprednisolone (MP) at 30 mg/kg/day for 3 days, followed by one week of reduced dosage to 1 mg/kg/day, then tapered off. Intravenous immunoglobulin (IVIG) was not given because it was unavailable. The patient was given aspirin at 80-100 mg/kg until his fever was controlled, then the dose was reduced to 3-5 mg/kg/day for 6 weeks. Following reduction of symptoms, the patient was discharged on the fifth day of hospitalization.

The second case was a 22-month-old girl from a private hospital who had been admitted with five days of high recurring fever, cough, rhinorrhea, conjunctivitis, and generalized erythematous rash.

Figure 1. Strawberry tongue (before and after therapy)

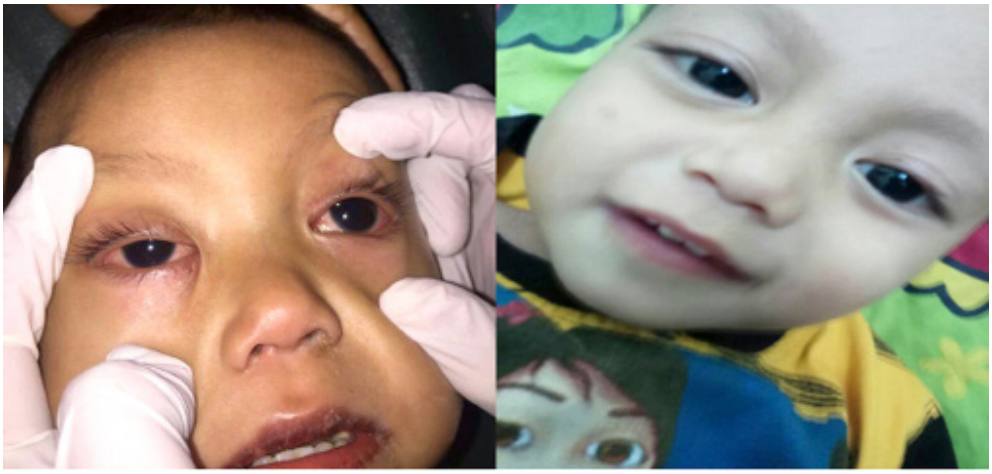

Figure 2. Conjunctival injection **(before and after therapy)**

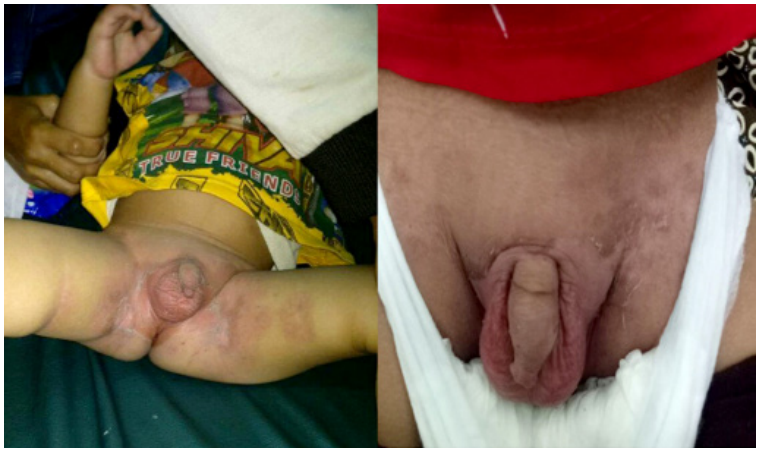

Figure 3. Perineal desquamation (before and after therapy)

She had been diagnosed with measles and discharged after three days. On the second week, the patient was referred to a pediatric cardiologist at Dr Sardjito General Hospital, Yogyakarta, due to desquamated skin all over her body and suspected KD. Laboratory investigations revealed that her leukocyte level was 15,230/μL, neutrophil 59.9%, and platelet count 629,000/μL. Her albumin level was 3.8 g/dL, and CRP was 128 mg/L. Echocardiography showed an aneurysm of the left coronary artery. The patient was treated with methylprednisolone (MP) at 30 mg/kg/day for 3 days and aspirin at 80 mg/day. IVIG was not administered because she was already in the second week of illness. The patient was discharged on the fourth day of hospitalization.

Discussion

Kawasaki disease is an acute, self-limited, febrile illness of unknown cause that predominantly affects children <5 years of age.[6] The disease is characterized by fever ≥ 5 days, bilateral bulbar non-exudative conjunctivitis, erythema and cracking of lips, strawberry tongue, and/or erythema of oral and pharyngeal mucosa, erythema and edema of the hands and feet in the acute phase, and/or periungual desquamation in the subacute phase, rash, and cervical lymphadenopathy.[6,7] In the presence of >4 principal clinical criteria, particularly when redness and swelling of the hands and feet are present, the diagnosis may be made with only 4 days of fever.[6]

The goal of therapy in the acute phase is to reduce inflammation and arterial damage and to prevent thrombosis in those with coronary artery abnormalities.[6] Glucocorticoids are classic anti-inflammatory agents that have been widely used for several pediatric diseases such as asthma, immunoglobulin A (IgA) vasculitis, nephrotic syndrome, leukemia, and collagen diseases.[8] Kawasaki disease is a form of inflammatory vasculitis, therefore, glucocorticoid therapy is an option for inhibiting inflammation. Although corticosteroids are the treatment of choice for other forms of vasculitis, its use has been controversial for children with KD. Intravenous methylprednisolone pulse (IVMP) therapy is often used to treat children with severe or refractory illnesses such as collagen vascular and renal diseases. However, corticosteroids have occasionally been used earlier as the second line therapy for patients unresponsive to initial IVIG treatment, as the routine first line therapy in combination with IVIG, or as the first line therapy in combination with IVIG for selected KD patients at high risk of unresponsiveness to initial IVIG.[9]

According to the 2017 American Heart Association (AHA) guidelines, single dose pulse methylprednisolone should not be administered with IVIG as routine primary therapy for patients with KD, and administration of a longer course of corticosteroids together with IVIG at 2 g/kg and acetylsalicylic acid (ASA) may be considered for treatment of high risk resistance IVIG patients with acute KD, when such high risk can be identified in patients before initiation

of treatment.[6] The addition of glucocorticoids to the initial therapy for KD has been shown to be most efficacious for Japanese children at high risk for IVIG resistance. A retrospective review found that treatment regimens that included prednisolone were associated with significantly shorter fever duration and a lower prevalence of coronary artery aneurysms.[10] Another study reported a lower incidence of coronary artery abnormalities and retreatment, shorter duration of fever, and more rapid decrease in CRP levels in the steroid group.[11] In contrast, a randomized study demonstrated that pulse intravenous methylprednisolone (30 mg/kg over 2 to 3 h) administered before IVIG in primary treatment did not improve coronary artery outcome or reduce the total days of hospitalization or fever.[12] In these patients, the following criteria can be used to select patients for treatment with glucocorticoids: enlarged coronary arteries at presentation (prior to IVIG treatment), age ≤12 months (and particularly age <6 months), KD associated with shock, and KD presenting with macrophage activation syndrome (MAS).[12]

Methylprednisolone therapy for Kawasaki disease in our two patients appeared to be beneficial and effective, but further studies are needed.

Conflict of Interest

None declared.

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