

# ASSOCIATION BETWEEN BLOOD LEAD LEVELS AND BASOPHILIC STIPPLING ERYTHROCYTES IN CHILDREN

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Abstract : Lead is the heavy metals that pollute the air and lead exposure continues to be the most serious public health problem. Increased lead absorption causes negative effect such us increased number of basophilic stippling erythrocytes. Battery smelting industry is an important source of lead pollutants today. Cinangka is one village in Bogor District that has battery smelting. Based on the results of investigation by KPBB (Komite Penghapusan Bensin Bertimbal) in 2010, the mean of blood lead levels (BLL) in Cinangka children's was 36.6 µg/dl (WHO threshold = 10 ug/dl).Aim of this study was to analyze the effects of BLL on the basophilic stippling erythrocytes in children. This study used a cross-sectional design. Blood samples were taken from 103 children in Cinangka Village Bogor District, Indonesia (2014) to measure BLL and basophilic stippling erythrocytes. A questionnaire used to determine the data on the level of parent's education, parent's income and nutrient intake. A nutritional status was known by calculating the Body Mass Index. Overall, 103 children (43 boys and 60 girls) with mean±SD age of 11,27±1,03 years were studied. The mean BLL on children was 14,70 µg/dl, whereas the lowest BLL 0,05 µg/dl and the highest BLL 52,11 µg/dl. The results also showed 61.2% of children had high BLL ( $\geq 10 \mu g/dl$ ). Statistical analysis with chi square showed that the BLL (p = 0.001) and mother's education level (p = 0.005) had significant association with basophilic stippling. Based on multivariate anaylisis, BLL was the most dominant variables associated with basophilic stippling.

Keywords: Blood lead levels (BLL), Basophilic stippling erythrocytes, children

## Introduction

Lead is one of the heavy metals that pollute the air and lead exposure continues to be the most serious public health problem (Mitchell, 2006). 40% of children in the world had blood lead levels > 5  $\mu$ g/dl. Some of them had blood lead levels > 10  $\mu$ g/dl of which 97% of them live in developing countries (Ustun, 2004). According to the WHO report, 18 children died in Thiaroye sur Mer, Senegal, during November 2007-March 2008 due to encephalopathy caused by lead intoxication. This could happen because of lead contamination to the environment resulting from the presence of informal companies/industry in the battery recycling/smelting (Haefliger, 2009).

The battery recycling/smelting industry is an important source of lead pollutants today. Most of the lead in the world is a secondary form as a result of battery recycling. 97% of the batteries in the world are reportedly recycled, most of which occur in low-income countries and generally informal industries, especially those that are not controlled by government (WHO, 2010).

Lead at the exposure level of at least 10  $\mu$ g/dl can interfere the hem synthesis, but in the initial disorder there is no clinical disorder (Lidsky, 2003). To compensate for the disruption of Hb synthesis by lead, medulla spinalis will increase the production of erythrocytes so that many reticulocytes and basophilic cells are obtained. The basophilic cell formed as part of the metabolic disorder of Hb formation is a sign of lead poisoning (Darmono, 2001). The Balali-Mood (2010) study in Iran found a significant association between

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blood lead levels and basophilic stippling erythrocytes (p = 0.048). A study by Kyrell in Turkey showed that children had higher blood lead levels than men and all women (p < 0.001). The results also showed that blood lead levels were negatively correlated with Hb in children (p < 0.05) (Kyrell, 2005).

A research conducted by Ahmad (2009) in Pakistan on 190 children aged 1-12 years divided into 2 groups where group A was the children living around the repair shop car as many as 62 people (39.5%) and group B was children who lived in the vicinity of a battery recycling of 128 people (60.5%). The results showed that mean of blood lead levels in children living around car repair shops and battery recycling sites were 11.4  $\mu$ g/dl (1.3-34.2  $\mu$ g/dl). Among the 190 children, 98 children (51.6%) had higher blood lead levels than the WHO recommended (10  $\mu$ g / dl). Among 98 children with high blood lead levels, 61 children (32.1%) had blood lad levels between 10-15  $\mu$ g / dl, 24 children (12.6%) had a blood lead level of 15-20  $\mu$ g / Dl and 13 children (6.8%) had blood lead levels> 20  $\mu$ g / dl indicating severe lead poisoning. Children living in areas near the battery recycling site (mean  $\pm$  SD 12.85  $\pm$  6.02) had high blood lead levels (p <0.020) than children living around car repair shops (mean  $\pm$  SD 10, 19  $\pm$  6.13) (Ahmad, 2009).

Cinangka is one of the villages in Bogor District which has battery smelting industries and becomes a place polluted by lead. In the 1980s, Cinangka became a center of former battery smelting where battery smelting activities became home-based industries. In the middle of the settlement, residents burn lead without chimneys and emissions drainage filters that cause polluted air and the sky is shrouded in black mist (Committee on the Elimination of Leaded Gasoline, 2011).

In Cinangka Village, lead pollution on the ground reached 270,000 ppm, whereas the WHO threshold is 400 ppm (Ministry of Environmental Health, Indonesia, 2011). Based on the investigation report of KPBB (2010) in Cinangka Village, it was known that the mean of blood lead levels of children above the WHO threshold (10  $\mu$ g/dl) and CDC threshold (5  $\mu$ g/dl) was 36.6  $\mu$ g / dl. The minimum BLL was 16.2  $\mu$ g/dl and maximum > 60  $\mu$ g/dl (Committee on the Elimination of Leaded Gasoline, 2011).

Children are groups that are vulnerable to lead exposure. Increased lead absorption cause a decrease of Hb, decrease in number of erythrocyte and shortening of the erythrocyte life span, an increase in the number of reticulocytes and an increase in the number of basophilic stippling erythrocytes (Joko, 1995). The objective of this study was to analyze the effects of BLL on the basophilic stippling erythrocytes in children.

## Method

This research used cross sectional research design. The study was conducted during May-June 2014. The population in this study were elementary school children grade 4, 5 and 6 in 4 elementary school located in Cinangka Village, Ciampea Subdistrict, Bogor Regency with the number of 535 students. Sampling was done by using simple random sampling method with total sample as 103 people.

Data on blood lead levels and basophilic stippling erythrocytes were obtained through venous blood sampling. Blood specimens were obtained by taking 3 ml of venous blood (2.5 ml for lead measurement and 0.5 ml for erythrocyte examination) in the arm/elbow fold and performed by 3 experienced health workers consisting of 1 laboratory staff Puskesmas Cilandak, South Jakarta which has been working for 20 years, 2 laboratory staff at Jati Rahayu Hospital, Bekasi who have been working for 10 years and 5 years. Measurement of blood lead levels used AAS (Atomic Absorption Spectrophotometer) at Hiperkes Laboratory, Jakarta. Basophilic stippling erythrocyte examination was done through examination of peripheral blood vessel preparation.

Questionnaires were used as instruments for measuring data on variable of nutrient intake, parent education level, parent income level and nutrient intake (protein, calcium and iron. Nutrient intake was obtained from the processing of food recall  $2 \times 24$  hours (on weekends and active days) then summed and made the average value, then the value is converted into percentage of nutrient intake by comparing the value with the standard set by the Ministry of Health RI no 75 of 2013 is appealed to the Indonesian nation. Meanwhile, for

nutritional status variables, obtained from the measurement of body height and weight of chuldren then calculated Body Mass Index (IMT) and converted into Z-score value.

Informed consent has been provided to the respondent to be considered whether willing or refusing to be a respondent. This research has been approved by the Ethics Committee of the Faculty of Public Health of the University of Indonesia.

Data analysis was done through univariate analysis, bivariate and multivariate analysis. To analyze the correlation of blood lead level, nutritional status, parent education level, parent income, nutrient intake, with basophilic branched erythrocyte was used chi square test. Multivariate analysis used multiple logistic regression.

## **Result and Discussion**

The sample of 103 children who were Cinangka elementary school students aged 9.33-14.00 years old with average age 11,27 years. Most of the respondents were female (58.3%). Respondents came from 4 different elementary schools located in Cinangka Village. Most respondents came from SD 3 Cinangka (31.1%). Most of the respondents were 5th graders (38.8%). Most respondents had high blood lead levels ( $\geq 10 \ \mu g / dl$ ) of 61.2% or 63 respondents (table 1).

Table 1. Distribution of Blood Lead Levels Categories Based on WHO Threshold

Variable	Frequency	Percentage
High Blood Lead Levels (≥10 µg/dl)	63	61.2%
Low Blood Lead Levels (< 10 $\mu$ g/dl)	40	38.8%
Total	103	100%

Laboratory results showed most respondents had basophilic stippling erythrocytes, with a percentage of 62.1% (table 2).

Variable		Frequency	Percentage
Bashopilic stippling	Yes	64	62.1%
erythrocytes	No	39	37.9%
Total		103	100%

Table 2. Distribution of Basophilic Speckled Erythrocytes in Children in Cinangka Village

The description of respondent characteristics consist of nutritional status, parent's education level, parent's income is shown table 3.

Table 3. Distribution of Nutritional Status, Parent's Education, Parents Income on Children in Cinangka

Variable	Frequency	Percentage
Nutritional Status		
- Stunting (BMI/Age < -3)	3	2.9
- Underweight (-3 $\leq$ BMI/Age < -2)	16	15.5
- Normal $(-2 \leq BMI/Age \leq 1)$	70	68.0
- Overweight (1 < BMI/Age≤ 2)	10	9.7
- Obesity $((-2 \le BMI/Age > 2))$	4	3.9

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Father's education level			
- Low	71	68.9	
- High	32	31.1	
Mother's education level			
- Low	81	78.6	
High	22	21.4	
Parent's income level			
- Low	79	76.7	
- High	24	23.3	

Most of the respondent (80%), percentage of the fulfillment of good nutrition intake of protein, calcium and iron under the standard of Nutrition Adequacy Ratio recommended by the Ministry of Healt wis 80%.

Bivariate analysis using chi square test between the main variables, covariable with dependent variable of basophilic stippling erythrocyte showed that high blood levels and low level of maternal education were risk factors for basophilic stippling erythrocytes (table 4).

Table 4. Relationship BetweenVariable Blood Lead Levels, Covariabel with Basophilic Stippling Erythrocyte	?
Variable on Children	

Variable	Basophilic stippling erythrocytes				Total			
	Yes		No				Odds Ratio (95% CI)	p value
	F	%	F	%	F	%		
Blood lead levels								
High ( $> 10 \mu g/dl$ )	60	95.2	3	4.8	63	100	180 (38.093-850.551)	0,001
Low ( $\leq 10 \mu g/dl$ )	4	10.0	36	90.0	40	100	- 180 (38.093-830.331)	0,001
Total	64	62.1	39	37.9	103	100		
Nutritional status			•					
Abnormal (BMI/Age >1 and BMI/Age <-2)	19	57.6	14	42.4	33	100	0,754 (0,324-1,757)	0,512
Normal (-2≤BMI/Age≤1)	45	64.3	25	35.7	70	100	_	
Total	64	62.1	39	37.9	103	100		
Father's education level			•					
Low	48	67.6	23	32.4	71	100	2,097 (0,900,4,906)	0.088
High	16	50.0	16	50.0	32	100	- 2,087 (0,890-4,896)	0,088
Total	64	62.1	39	37.9	103	100		
Maternal education level								
Low	56	69.1	25	30.9	81	100	2 020 (1 450 10 522)	0,005
High	8	36.4	14	63.6	22	100	3,920 (1,459-10,532)	
Total	64	62.1	39	37.9	103	100		
Parent income level								

	1		1			1	1	1
Low	52	65.8	27	34.2	79	100	1,926 (0.763-4.859)	0.162
High	12	50.0	12	50.0	24	100	1,920 (0.703-4.039)	
Total	64	62.1	39	37.9	103	100		
Protein intake								
< 25.715	10	83.3	2	16.7	12	100	$2500(0.002 \pm 0.18)$	0.072
≥ 25.71%	54	59.3	37	40.7	91	100	2.500 (0.903-6.918)	0.072
Total	64	62.1	39	37.9	103	100		
Calcium intake								
< 6.28%	20	76.9	6	23.1	26	100	$2500(0.002 \pm 0.18)$	0.072
≥ 6.28%	44	57.1	33	42.9	77	100	2.500 (0.903-6.918)	0.072
Total	64	62.1	39	37.9	103	100		
Calium intake								
< 10.67%	34	66.7	17	33.3	51	100	1 467 (0 659 2 267)	0.348
≥10.67%	30	57.7	22	42.3	52	100	1.467 (0.658-3.267)	0.548
Total	64	62.1	39	37.9	103	100		
Iron intake								
< 24.00%	34	68.0	16	32.0	50	100	1 (20 (0 728 2 (44)	0.222
≥24.00%	30	56.6	23	43.4	53	100	1.629 (0.728-3.644)	0.233
Total	64	62.1	39	37.9	103	100		

After bivariate analysis, there were 7 variables that can enter into the multivariate model. These variables include blood lead levels, father's education level, maternal education level, parent's income level, protein intake, calcium intake and iron intake. The final model of multivariate logistic regression analysis showed that blood lead levels were the dominant variable associated with basophilic stippling erythrocytes (OR = 180.00, 95% CI: 38.093-850,551). The calculation result of multivariate anaylisis showed OR = 18,729. The end result of the interaction test showed an interaction between the variables of blood lead levels in the blood with protein intake (OR = 138.267, 95% CI: 31,113-614,457). The results of the confounding test was found that the variable of iron intake and maternal education was the confounding variable for the correlation of blood lead level with basophilic stippling erythrocytes.

## Discussion

The mean of blood lead level of the respondents was 14.70  $\mu$ g / dl, higher than the WHO threshold (10  $\mu$ g / dl). In 2012, the CDC has set a threshold level of blood lead level of 5  $\mu$ g/dl (PEHSU, 2013). The lowest blood lead levels was 0.05  $\mu$ g/dl and the highest was 52.11  $\mu$ g / dl.

The results of Tiurdinawaty's research on elememantary school students grade 4 and 6 in Cikarang subdistrict in 2008 showed the average of blood lead was lower than the mean of blood lead level in Cinangka elementary students in this study. The mean blood lead level in elementary school students in Cikarang was  $6.13 \ \mu g/dl$ , where the median value was  $5.72 \ \mu g/dl$ . Lowest value  $0.17 \ \mu g/dl$  and the highest  $15.97 \ \mu g/dl$ (Tiurdinawaty, 2008). This showed that children in Cinangka have been exposed to higher lead due to environmental contamination from the battery smelting industry that has been established since the 1980s. The results of Ahmad's research in Pakistan showed that children living around car repair shops and batteries recycling sites have an average blood lead level of  $11.4 \ \mu g/dl$  ( $1.3-34.2 \ \mu g / dl$ ). Children living in areas near the battery recycling site (mean = 12.85) had high blood lead levels (p <0.020) compared with children living around car repair shops (mean = 10,19) (Ahmad, 2009).

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Based on the information in the Cinangka, it was known that several factors related to the high blood lead level on children in Cinangka, especially children with blood lead level  $\geq 30 \ \mu g/dl$ . It could happen because the parents of their chidren working as laborers or employees in the battery smelting industry, the residence of children closed to battery smelting industry, ( $\pm 1$  km distance), as well as children residence near the river where washing used batteries.

According to CDC (2012), blood lead levels in children depend on the environment, habits and nutritional status Nutritional status is also related to the daily intake of nutrients in children. In low-calcium, iron, zinc and protein dietary conditions, can increase lead absorption14 (PEHSU, 2013).

According to Caroline W. (1995) in Suciani (2007), blood lead levels can be affected by prolonged exposure, exposure dose and lead entry into the body. The level of lead in the blood is also affected by the distance between the dwelling and the source of the pollutant17. The research of Chahaya et al (2005) in Pematang Siantar City found that there was a correlation between lead level in blood of pedicab driver with the distance of house to source of pollutant18. According Atrisman (2002), ambient air with a 0.5 meter radius of the source of exhaust emissions is the location of the greatest risks. Distance of 0.5-1 km is a moderate risk and above 1 km is a mild risk.

Of the 103 children in Cinangka Village who became the sample, most of them had basophilic spotted erythrocytes (62.1%). The result of bivariate analysis was statistically known that there was correlation between blood lead levels with basofilic stippling erythrocytes. Similar results are shown by the Balali-Mood (2010) study in Iran. The study found a significant association between blood lead levels and basofilic stippling erythrocytes (p value = 0.0487).

In the erythrocyte maturation process, lead causes deficiency of the pyrimidine-5'-nucleotidase enzyme (this P5'N enzyme is involved in the degradation of RNA / ribosomal ribonucleic acid in reticulocytes). Due to P5'N enzyme deficiency an intracellular aggregate is formed as a consequence of ribosomal degradation failure. The presence of basophilic spots in these erythrocytes is a morphological form of intracellular aggregates due to the failure of ribosomal degradation. Genetically, P5'N enzyme deficiency is associated with chronic hemolysis, characterized by basophilic stippling in erythrocytes and accumulation of pyrimidine nucleotides (Rodak, 2012 and Greer, 2014). The basophilic cell formed as part of the metabolic disorder oh Hemoglobin (Hb) disorder of Hb formation is a sign of lead poisoning (Darmono, 2001)

The presence of basophilic stippling erythrocytes indicates that lead poisoning has occurred on children in Cinangka. Most children with lead poisoning are asymptomatic. There were 3 children in Cinangka who had blood lead levels > 40  $\mu$ g / dl. This indicates that the children has been severely exposed by lead despite the undetectable signs of poisoning (Ministry of Health Indonesia, 2013).

Based on the study, nutritional status and nutrient intake (protein, calcium and iron intake) was not significantly correlated with basophilic stippling erythrocytes on children in Cinangka. Indirectly the nutritional status affects the basophilic stippling erythrocytes by absorption of lead levels in the blood. Children with poor nutritional status are more vulnerable to absorb more lead than children with good or normal nutritional status.

The existence of the relationship is not significant, it is likely to indicate that there are other factors that more influence the occurrence of basophilic stippling erythrocytes. Most likely is due to environmental or residential factors. A child with the same nutritional status but residing in different environments (such as near or remote home locations with the battery industry), is likely to receive different lead exposures. Therefore, it can increase the risk of accumulation of lead in the body and cause basophilic stippling in their erythrocytes.

As stated by CDC (2012) that environmental, habits and nutritional status can influence the variation in lead levels in a child's blood (PEHSU, 2013). Nutritional status is also related to daily nutritional intake in

children. When the intake of certain nutrients is less then there will be nutritional deficiency. Based on Correia (1998) in Suciyani (2007) on low calcium, iron, zinc and protein diets can increase lead absorption (Suciani, 2007). High lead absorption further increases the risk that a person has basophilic spotted erythrocytes.

The result of bivariate analysis showed that there was not significant correlation between father education level and basophilic stippling eytrhrocytes on children in Cinangka. A person's level of education basically affects one's knowledge. Although knowledge is part of the area of conduct, it will not guarantee that a person with sufficient knowledge possesses the same behavior (Syafri, 2013).

However, the results showed that children who had mothers with low educational level were 3.92 times more likely to have basophilic-stippling erythrocytes than children with mothers with higher education (p = 0.005, 95% CI: 1.459-10.532). Maternal education will affect knowledge as well as parenting patterns at home. In contrast to the level of education of the father who did not show a significant relationship, maternal education showed a significant relationship. This means that mothers with higher education are able to adopt a healthy lifestyle for themselves and their families so as to minimize the adverse effects of lead hazard, whether through good dietary regulation or other prevention efforts. The rigorousness of this study is the non-measurement of maternal health behavior.

The statistical analysis showed there was not relationship between parent's income level and basophilic stippling erythrocytes on children in Cinangka. This can be caused even if a family has a high income and is able to meet the needs of his life well, but possibly because of ignorance or lack of knowledge and awareness about the pattern of good consumption of food then causes the family to consume insufficient food types of certain nutrients and less variation in diet.

Theoretically, families with high income levels will be better able to provide for their daily needs, including the need for food, whereas with a low monthly household income the possibility of a child and other family members not getting food rich in sufficient iron and the diet of children living in families with less economics are usually monotonic (Gutema, 2014).

Friedman's research showed that relatively well-earned family meals are not much different in quality compared to low-income family meals. This suggests that ignorance of the benefits of food for health is one of the reasons for the lack of nutrient quality consumed (Syafri, 2013). Research conducted by Zhou, et al in Adelaide, Australia in 2005-2007 did not find any association of socioeconomic status with macro nutrient intake nor micro (Zhou, 2012).

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