

Effect of Calcium Supplementation on School Children's Blood Lead Levels in Indonesia

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Abstract: Population exposures to lead are mainly related to: vehicle exhaust, in areas where leaded-gasoline is still being used; paint and lead solder in canned food; drinking water pipes; and from certain culturally-based exposures, such as use of lead-glazed ceramics or traditional medication. It is well documented that excess exposure to lead causes health problems in children including neurobehavioral deficits. The objective of this study was to examine the effect of daily calcium supplementation for a three months period on reducing blood lead levels (BLLs) among elementary school children aged 9 to 11 years old in Bandung, Indonesia. Forty elementary schools were randomly selected, 400 children enrolled, and a total of 298 children completed follow-up of 3 months period. Schools were divided randomly into two groups of calcium-supplement intervention groups (250 mg and 500 mg) and one control group with no intervention. Capillary blood samples were analyzed for lead levels before and after three months of calcium supplementation. Paired-t test, ANOVA, and multivariate risk factors models using Generalized Estimating Equations (GEE) were performed. The percent reduction in BLLs for each group was: 15.1% (no calcium) from the baseline of 13.7 μ g/dL; 49.8% (250 mg Ca) from the baseline of 14.8 μ g/dL; and 74.5% (500 mg Ca) from the baseline of 14.1 μ g/dL. This study found statistically significant inverse associations with dietary Ca supplementation and BLLs in young children. These findings suggest that calcium supplementation effectively lowers BLLs in school children.

Keywords: children blood lead levels, calcium supplement intervention, air pollution

1. Introduction

Lead (*Plumbum*, Pb) is generally still being used as a gasoline additive to increase octane in Indonesia. Pb is also still used in industrial processes, such as: battery, printing, solder, textile, and other manufacturing. The largest source of Pb pollution in an environment (~80%) is due to the use of leaded gasoline and the rest from industrial pollution and household sources including: polluted water, food canning, and domestic waste such as solders, and others (CDC, 1991).

The plan for elimination of leaded gasoline was scheduled through Ministry of Mining and Energy (MoME) for Jakarta in 2001, all islands of Java in 2002, and all over Indonesia in 2003. The program has been implemented in Jakarta; however, removal of lead from gasoline has not been implemented in other provinces for many reasons. In 2002, MoME rescheduled the elimination of leaded gasoline for completion in 2005. Finally, the phase-out of leaded gasoline was successfully implemented in July 1st, 2006 in Indonesia, and leaded-gasoline is no longer sold.

In many countries, the effect of Pb on health has been considered an important basis for eliminating leaded gasoline. There is a scientific need of proving the effect of Pb exposure in environment on health problems in Indonesia, especially children, so as to make decision makers aware on the reduction of Pb in environment control. Several studies on Blood Lead Levels (BLLs) in Indonesia indicate a high potential health risk among the target populations studied. In 1987, drivers in urban Jakarta had BLLs twice as high as those living in rural areas. In 1998, it was estimated that about 74% of people tested living in heavy transportation areas in Jakarta had BLLs more than 30 μ g/dl (Tugaswati, 1998). In 2001, a study among elementary school children in Jakarta indicated that 35% had BLLs

more than 10 μ g/dl (Albalak et al., 2003). Four years after Jakarta was supposed to be free from the use of leaded gasoline, 1.3% of elementary school children still indicated BLLs more than 10 μ g/dl (Haryanto, 2005).

Calcium, which can be obtained from milk consumption, milk product, and nutritional supplements, is an essential nutrient for bone health and other functions, especially for children, pregnant, nursing, and menopausal women. Calcium may be able to provide protection against Pb exposures by reducing Pb absorption in human body (Meredith et al, 1977). Several international studies on animal trials showed the existence of competition of Pb and Ca in the absorption process of intestines (Barton et al, 1978). Epidemiological studies on children indicated a similar finding that an intake of Ca related to level of BLLs. Children who have less daily consumption of Ca tend to have more Pb absorption from the environment in comparison to those who have enough daily Ca consumption (Chen and Xiaobin, 2001). Maternal diet during pregnancy, higher intakes of calcium, iron, and vitamin D were found to have association with lower neonatal blood lead levels (Schell, et al. 2003).

The overall objective of this study was to identify the effects of calcium supplementation on reducing blood lead levels of school children in Bandung, Indonesia. We hypothesized that daily calcium supplementation for three months could reduce blood lead levels and that these reductions would be larger among those children who consumed 500 vs. 250 mg calcium supplement per day. We also sought to determine factors that contributed to the reduction in blood lead with calcium consumption.

2. Methods

2.1 Study Design and Population

This intervention study employed a randomized community trial design that was completed in elementary schools in Bandung, Indonesia between August 2005 and December 2006. Bandung city was chosen for the study based on its risk to the high exposure of air pollution. As the 2nd largest metropolitan area in Indonesia with a population of 7.4 million in 2007, Bandung is located 768 metres (2,520 ft) above sea level, and lies in a river basin surrounded by volcanic mountains. Its daily wind speed (2 knots) does not support air circulation and purification in this area.

A total of 400 school-children between the ages of 9 and 11 years old were enrolled from 40 schools. Randomization constructed from the 40 schools in which 14 schools (140 students) selected as intervention group of 500 mg Ca/day, 13 schools (130 students) selected as intervention group of 250 mg Ca/day, and 13 schools (130 students) selected as non intervention group. All respondents were followed for three months (Figure 1) between June and December 2006. Three hundred thirty-six students from 35 schools completed the intervention phase of the study. Of those, 298 participants had complete data on blood lead levels before and after the intervention and are included in the analyses. Most of the loss to follow children (64 students) was caused by not having permission from the school principals due to examination week, school celebration, and new management at the school. Meanwhile, lack of blood lead results (36 blood samples) was caused by a very high humidity at the place of measurement, which was affected to the performance of the in situ equipment (not all of schools had air-conditioned room for blood samples analysis).

Two phases of sampling selection were employed. First, a cluster sampling design with proportionate probability to size (Lwanga and Lemeshow, 1998), based on the number of children in each school, a total of 40 elementary schools out of 962 schools in Bandung city were selected. Also, based on an estimated prevalence of blood lead levels $>10 \mu\text{g}/\text{dl}$ in elementary school children (35% from a previous study in Jakarta), the sampling error of 10% with two design effects, and 95% confidence limit, the minimum sample required was 176 elementary school children and a sample size of 400 school children was used. Since there are 40 clusters (schools) of elementary school children, it meant that each cluster needed 10 children (5 each in the 3rd and 4th grades). Second, sampling formula of hypothetical test for two population's means (Lwanga and Lemeshow, 1998) is used to randomly divide schools into three groups of interventions (500 mg Ca/day; 250 mg Ca/day; no supplement). A survey of BLLs of elementary school children in Jakarta 2001 indicated that BLLs were (mean (SE)) 8.6 (3.8) $\mu\text{g}/\text{dl}$ (Albalak et al, 2003). Assuming a 10% difference in average BLLs before and after intervention and a confidence limit of 95%, the minimum sample was 62 children for each intervention group.

Calcium supplement used was chewable tablet with chocolate taste of Calcium Sandoz Junior produced by Novartis Inc. Bogor, Indonesia (under licensed of Novartis

Inc. International Switzerland). One tablet supplement contains of 625 mg calcium carbonate (equivalent with 250 mg calcium ion). Four parties were closely involved to reminding participants in taking the supplement daily, they are participants' parents, teacher, participants to his/her parents, and field enumerator for keeping the compliance. Enumerators monitored compliance by contacting parents and teacher every 3 days using Short Message System or telephone.

The Faculty of Public Health University of Indonesia Review Board approved the study. All parents were explained the study procedures and provided written informed consent before agreeing to participate the study.

2.2 Data Collection

Blood lead levels were measured at baseline and following the 3-month intervention using the Portable Lead Care Analyzer (ESA, Inc. Chelmsford, MA). Blood-lead was drawn by a medical doctor at the health room of the school from the subject's soap-washed finger by using a disposable and sterile lancet. Demographic characteristics of participants were collected by structured questionnaires. Nutritional status of participants was collected by anthropometric method and measurement of height by ages: 1) Z-score more than 2 Standard Deviation (SD) = over; 2) Z-score between -2 and 2 SD = normal, and; 3) Z-score less than -2 SD = under. Frequency of consumption of calcium-rich foods was measured using a modification of food frequency questionnaire (FFQ) before and after the intervention (Fikawati et al 2005).

2.3 Data Analysis

Paired t-tests were employed to identify the difference of BLLs between pre- and post-intervention within groups. A test of one-way ANOVA and posthoc Bonferroni were employed to identify means difference of BLLs among groups. The proportion of BLLs $\geq 10 \mu\text{g}/\text{dl}$ (CDC, 1997; CDC, 1991) before and after the intervention and percent reduction in blood lead by intervention group was calculated.

A multiple regression analysis using Generalized Estimating Equations (GEE) was employed to identify the effect of Ca supplement on BLLs reduction after controlling for other variables and to obtain a prediction model of other risk factors on BLLs. Independent variables in multivariate analysis included: Ca supplement group; frequency of consumption of Ca-rich foods; nutritional status; transportation used to school; time to get to school; distance of school to home; and home location proximate to main road. Further analysis using step-wise GEE regression models to exclude variables with p-value >0.05 from the final model.

3. Results

3.1 Characteristics of Respondents

Of the total 400 participants at baseline, 298 participants (74.5%) completed the study involving 103 (25.7%) students

in 500 mg Ca /day group; 93 (23.3%) students in the 250 mg Ca/day group; and 102 (25.5%) students in the group without Ca supplement (Table 1). The 25.5% of participants who did not complete the study were evenly distributed among the groups, with no difference in characteristics from those participants who finished the study. The three groups were approximately equal proportions of male and female students except in the 250 mg group which had more female students ($p=0.04$). Most children walked to school as their primary mode of transportation followed by car or public transportation and motorbike. Most children traveled between 200 to 1000 meters to school which took them between 5-30 minutes of travel time. The large majority of participants in all three groups were from the Kampong slum area of the city.

3.2 Consumption of Calcium-rich Foods

Dietary consumption with respect to calcium-rich foods was measured two times, at the beginning and at the end of data collection activities, using the FFQ and there were no significant differences over time. At both time points, a similar pattern of Ca-rich food consumption was observed. Overall, most respondents did not consume Ca-rich food often. Table 2 shows the baseline intake of ten types of Ca-rich foods (biscuits, meat, milk powder, fresh milk, sweet milk, tofu, tempeh, haricot (beans), chocolate, ice cream) by intervention group.

3.3 Distribution of BLLs

Before the intervention, mean (SD) BLLs were: 14.1 (8.10) $\mu\text{g/dL}$; 14.8 (7.5) $\mu\text{g/dL}$; and 13.7 (7.5) $\mu\text{g/dL}$ in the 500 mg Ca/day; 250 mg Ca/day; and no supplement group, respectively (Table 3). There was no statistically significant difference in BLLs between the three groups before the Ca supplement intervention ($p>0.05$). However, after the three month intervention, the three groups experienced an average reduction in BLLs of: 7 ($p<0.0001$), 6.1 ($p<0.0001$), and 1.6 ($p=0.02$) $\mu\text{g/dL}$, respectively. At baseline, 65.7% of participants had higher BLLs than the CDC level of concern in children ($\geq 10 \mu\text{g/dL}$) (CDC, 1991). The proportion of BLLs $\geq 10 \mu\text{g/dL}$ before and after the intervention were: 68.5% and 17.5% (500 mg Ca/day); 66.4% and 33.3% (250 mg Ca/day); and 62.3% and 52.9% (no supplement) (Table 4). This corresponds to a 74%, 49.8%, and 15.1% reduction in the high (500 mg), medium (250 mg) and no calcium groups, respectively.

4. Discussion

In this study, before the intervention there were no significant differences in the average of BLLs between the intervention groups ($p>0.05$). The average of BLLs observed in this study (14.1 $\mu\text{g/dL}$) was higher than found by CDC's Jakarta study (4.2 $\mu\text{g/dL}$ in 2005 and 8.6 $\mu\text{g/dL}$ in 2001).

Relatively low dietary Ca intake of children in Bandung, at approximately 55% of adequate intake, was similar to daily Ca intake in a group of youth in Bogor, Indonesia (Syafiq and Fikawati, 2004; Fikawati et al, 2005). In the U.S., NHANES III data showed a similar finding that almost 60%

of children age of 4-8 years have regular dietary intake of Ca lower than 800 mg/day (Bruening et al, 1999).

Three months of dietary calcium supplementation was found to reduce blood lead levels among children. The findings indicate that the consumption of Ca 250 mg/day and 500 mg/day was able to reduce BLLs on average by 49.8% and 74.5%, respectively. The group without Ca intervention showed an average reduction of 15.1%, which may be due to reduction of Pb exposures in the environment or changing behavior patterns over the study period. The proposed discontinuation of adding tetra-ethyl lead into gasoline as of July 2006 may be considered as one of the possibilities for reduced exposure opportunities.

In the group without Ca supplementation, the percent reduction in BLLs over the study period was 15.1% in comparison to 49.8% and 74.5%, respectively, in the 250 and 500 mg/day Ca supplement groups. One limitation of this study is that there is no data available when compliance was monitored by contacting parents and teacher. However, these results suggest that the Ca supplement intervention was responsible for the decline in BLLs over the study period. These results are supported by several previous studies, including both animal and human data.

Increased absorption and retention of lead by calcium-deficient animals has been observed (Six and Goyer, 1970; Mahaffey et al., 1973; Barton et al, 1978). Confirmation of the impact of low dietary calcium intakes has also been found among human subjects who were also shown to have increased lead absorption when their diets were low in calcium (Heard and Chamberlain, 1982). Several observational studies have indicated that there is a pattern of reduction in BLLs for those people who have adequate dietary Ca intake. In the NHANES II (1976 – 1980), higher reported Ca intake was related to significantly lower with BLLs in U.S. children age 3 - 11 years old (Mahaffey et al, 1986). Lacasana et al. (2000) observed a statistically significant association between daily calcium intake and blood lead levels in children. In an observational study of maternal diet during pregnancy, higher intakes of calcium, iron, and vitamin D were associated with lower neonatal blood lead levels (Schell, et al. 2003).

A randomized clinical trial of calcium-supplemented infant formula to prevent lead absorption was carried out in the U.S. This study found that infants on calcium-supplemented formula had lower increases in BLLs at four months, but this effect was not sustained to 9 months of age (Sargent et al, 1999). Another randomized trial of Ca supplementation in the U.S. among children aged 1 to 6 years with BLLs 10-45 $\mu\text{g/dL}$ found no effect of Ca supplementation on reduction in BLLs over the study period (Markowitz et al. 2004). This study aimed to provide 1,800 mg Ca/day through a combination of diet and supplementation and doses were adjusted weekly to achieve the desired amounts for each child.

Two large randomized clinical trials have been conducted to assess whether calcium supplements reduce blood lead levels during pregnancy and lactation and the overall findings suggest that calcium supplementation is associated

with modest reductions in blood lead levels when administered during pregnancy or lactation. In a randomized, double-blind, placebo-control trial of calcium supplementation during lactation, Hernández-Avila et al. (2003) showed that 1200 mg daily dietary supplementation with calcium carbonate among lactating women reduced maternal blood lead levels 15% - 20% over the course of lactation. Compared with women who received the placebo, those who took supplements had a modest decrease in their blood lead levels of $-0.12 \mu\text{g/dL}$ at 3 months (95% CI = -0.71 to $0.46 \mu\text{g/dL}$) and $-0.22 \mu\text{g/dL}$ at 6 months (95% CI = -0.77 to $0.34 \mu\text{g/dL}$). In another randomized control trial, calcium supplementation (1,200 mg) was associated with modest reductions in blood lead when administered during pregnancy. During the 2nd and 3rd trimesters of pregnancy, calcium supplementation (1,200 mg) was associated with an average reduction of 19% in blood lead concentration in relation to placebo ($p < 0.001$) (Ettinger, et al. 2009). These effects were strongest in the most compliant women, including those who: consumed $>75\%$ pills (-24% , $p < 0.001$); or had baseline blood lead greater than $5 \mu\text{g/dL}$ (-17% , $p < 0.01$) In the subset of most compliant women with high patella bone lead ($>5 \mu\text{g/g}$) and reported use of lead-glazed ceramics, the reduction in blood lead of 31% corresponded to an average reduction of $1.95 \mu\text{g/dL}$ (95% CI = -0.78 to -2.87). As indicated by the studies in pregnancy and the postpartum period, it is possible that the major calcium effects on lead absorption and distribution occur when dietary calcium is deficient (Hertz-Picciotto et al., 2000) and high levels of calcium intake may be needed to compensate (Johnson, 2001).

In summary, this study found statistically significant inverse associations with dietary Ca supplementation and BLLs in young children. This finding indicates that daily intake of Ca supplement for 3 months reduces BLLs in children. Intake of Ca supplement of 500 mg/day for 3 months reduced more BLLs than intake of Ca supplement of 250 mg/day, however, it is unclear if other dosing regimens or longer periods of supplementation would have had larger effects. Nonetheless, development of a routine nutritional intervention program for consumption of Ca-rich foods, and possibly the addition of Ca supplements, may be prudent particularly in areas where dietary Ca intake is low and Pb exposure in the environment continues.

5. Acknowledgement

I would like to thank for all of the supports given to this study especially to PT. Novartis Indonesia, United States Asian Environment Partnership, City of Bandung Health Office, All of the involved Elementary Schools in Bandung, Field coordinator and Data collectors.

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Table 1: Baseline characteristics of participants by intervention group

Characteristics of participants	Intervention Group (%)			p*
	Without Ca (n = 102)	250 mg (n = 93)	500 mg (n = 103)	
Sex				
Male	42.3	36.9	49.3	0.04
Female	57.7	63.1	50.7	
Transport to school				
Walk	70.8	44.6	51.0	0.43
Bike	0.0	0.0	11.0	
Motorbike	11.5	34.7	14.0	
Car/public transport	17.7	20.7	24.0	
Distance to school				
< 200 m	26.0	11.9	19.8	0.01
200 – 500 m	38.5	31.7	29.7	
501 – 1000 m	20.8	25.7	16.8	
> 1 km	14.7	30.7	33.7	
Duration to school				
< 5 minute	28.1	9.8	24.8	0.06
5 – 15 minute	44.8	73.5	48.5	
16 – 30 minute	18.8	13.7	18.8	
> 30 minute	8.3	3.0	7.9	
Home location				
Main road	8.6	12.7	8.1	0.90
Cluster housing	17.2	22.6	36.4	
Kampong/Slum area	74.2	64.7	55.5	
Nutritional status†				
Over	2.0	0.0	0.0	0.03
Normal	72.7	88.0	91.7	
Under	25.3	12.0	8.3	
Hemoglobin				
<12 g/dl	4.6	2.9	5.4	0.75
≥12 g/dl	95.4	97.1	94.6	

* p-value from ordered logit regression

† Nutritional status defined as: over (Z-score 2 SD); normal (Z-score -2 to 2 SD); under (Z-score <-2 SD)

Table 2: Frequency of calcium-rich food consumption at baseline, by intervention group

Calcium-rich Food Type	Without Ca n=102 (%)	Ca 250 mg n=92 (%)	Ca 500 mg n=103 (%)	p*
Biscuits Sometimes	53 (51.0)	48 (54.3)	60 (56.6)	0.91
Often	49 (49.0)	44 (45.7)	43 (43.4)	
Meat Sometimes	78 (77.1)	38 (42.6)	51 (48.1)	0.00
Often	24 (22.9)	54 (57.4)	52 (51.9)	
Milk, powder Sometimes	75 (78.1)	60 (60.6)	78 (73.6)	0.03
Often	27 (21.9)	32 (39.4)	25 (26.4)	
Milk, sweet Sometimes	53 (51.7)	49 (52.4)	62 (59.3)	0.99
Often	49 (48.3)	43 (47.6)	41 (40.7)	
Milk, fresh Sometimes	70 (67.7)	65 (71.3)	46 (45.3)	0.89
Often	32 (32.3)	27 (28.7)	57 (54.7)	
Tofu Sometimes	88 (85.4)	50 (56.4)	70 (67.0)	0.00
Often	14 (14.6)	42 (43.6)	33 (33.0)	
Tempeh Sometimes	85 (83.3)	52 (58.5)	72 (67.9)	0.00
Often	17 (16.7)	40 (41.5)	31 (32.1)	

Haricot Sometimes	65 (65.6)	52 (54.3)	52 (50.9)	0.19
Often	37 (34.4)	40 (45.7)	51 (49.1)	
Chocolate Sometimes	76 (79.2)	56 (58.5)	90 (83.0)	0.00
Often	26 (20.8)	36 (41.5)	18 (17.0)	
Ice cream Sometimes	76 (81.2)	74 (76.6)	85 (77.4)	0.79
Often	26 (19.8)	18 (23.4)	23 (22.6)	
All food Sometimes	65 (65.5)	51 (51.8)	40 (38.8)	0.00
Often	37 (34.5)	46 (48.2)	63 (61.2)	

* p-value from ANOVA comparing three groups

Table 3: Difference in blood lead levels before and after intervention

Intervention Group	Blood Lead Levels (µg/dl)		p-value*
	Before Intervention†	After Intervention	
	Mean(SD)	Range	
500 mg Ca	14.1 (8.1)	1.5-56.1	7.1 (2.7) 2.6-15.0 <0.0001
250 mg Ca	14.8 (7.5)	3.3-38.2	8.7 (4.5) 0.0-38.2 <0.0001
Without Ca	13.7 (7.5)	2.2-60.0	12.1 (6.8) 3.6-42.8 0.02

* p-value from the paired t-test for difference before and after intervention calculated for each group separately
 † There was no statistically significant difference in BLLs between the three groups before the Ca supplement intervention (p>0.05).

Table 4: Proportion of blood lead levels ≥10 µg/dl before and after intervention, and percent reduction in blood lead, by intervention group

	Intervention Group			p-value
	Without Ca (N=102)	250 mg (N=93)	500 mg (N=103)	
Proportion of BLL ≥10 µg/dl				
Before Intervention	62.3%	66.4%	68.5%	0.295
After Intervention	52.9%	33.3%	17.5%	
Percent Reduction in Blood Lead				
[(before-after)/before]*100	15.1%	49.8%	74.5%	<0.001

Table 5: Final model of multivariate GEE* (N=298)

	Reduction of BLLs	
	Coefficient	p
Constant		
Intervention Group		
Without Ca	14.94	0.00
250 mg Ca	1.00	0.04
500 mg Ca	-2.36	0.01
Nutritional Status		
Good/Normal	-2.47	
Low	1.00	0.17
Time of trip to school		
< 5 minutes	1.17	
5-15 minutes	1.00	0.24
16-30 minutes	1.28	0.48
> 30 minutes	-0.29	0.64
Phase of data collection		
Before intervention	-0.63	
After intervention	1.00	0.00
	-4.97	

Wald chi2(6) = 105.4

* GEE model accounting for repeated measures and also clustering by school