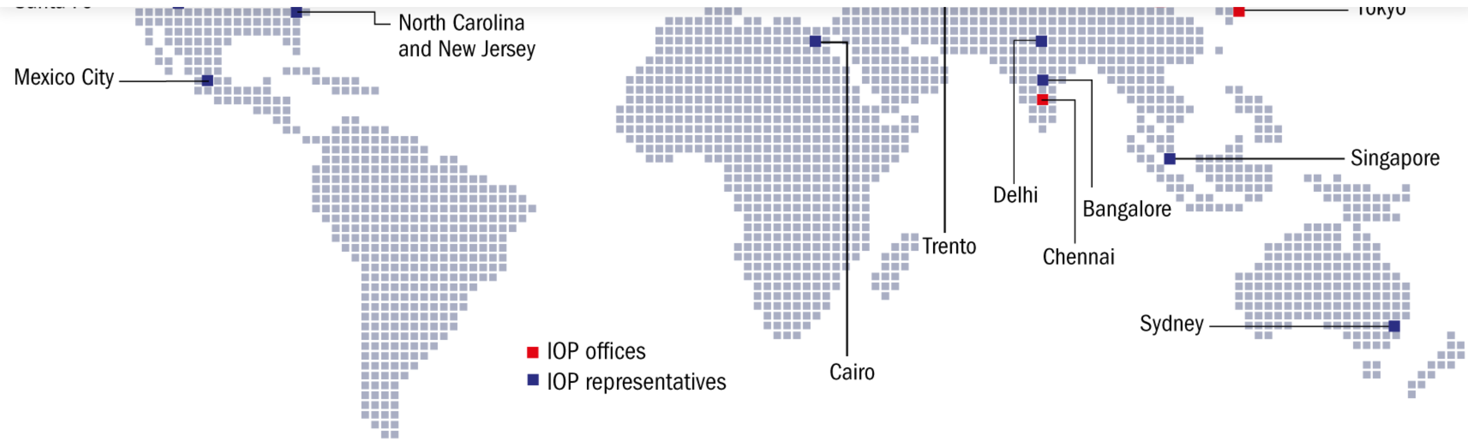

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Low zinc serum levels and high blood lead levels among school-age children in coastal area

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Abstract. The coverage of environmental lead toxicant was quiet wide. Lead exposure recently has been expected to be associated with zinc deficiency and blood indices disturbance. Emphasizing on children, which could absorb more than 50 % of lead that enters the body. Lead became the issue on the coastal area due to it has polluted the environment and waters as the source of fisheries products. This was a cross sectional study to determined nutritional status, blood lead levels, zinc serum levels, blood indices levels, fish intake among school children in coastal region of Semarang. This study was carried out on the school children aged between 8 and 12 years old in coastal region of Semarang. Nutritional status was figured out using anthropometry measurement. Blood lead and zinc serum levels were analyzed using the Atomic Absorbent Spectrophotometry (AAS) at a wavelength of 213.9 nm for zinc serum and 283.3 nm for blood lead. Blood indices was measured using auto blood hematology analyzer. Fish intake was assessed using 3-nonconsecutive days 24-hours food recall. The children had high lead levels (median 34.86 μg/dl, range 11.46 - 58.86 μg/dl) compared to WHO cut off. Zinc serum levels was low (median 18.10 μg/dl, range 10.25 – 41.39 μg/dl) compared to the Joint WHO/UNICEF/IAEA/IZiNCG cut off. Approximately 26.4% of children were anemic. This study concluded that all school children had high blood lead levels, low zinc serum, and presented microcytic hypochromic anemia. This phenomenon should be considered as public health concern.

Keywords: blood lead, zinc serum, coastal region, microcytic hypochromic anemia, school children

1. Introduction

Zinc (Zn) is an essential trace element plays role as a cofactor of more than 100 metaloenzym, plays an important part in cell regeneration, metabolism, growth and immune function [1]. Zinc deficiency is associated with suboptimal growth, diarrhea, and decreased of immunity [2]. *World Health*

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Organization (WHO) estimates the prevalence of zinc deficiency in the world's population is ranged between 4 and 73%. Additionally, about 5 to 30% zinc deficiency has occurred in children and adolescents both in developed and developing countries [3]. Especially in the developing countries, harmful environmental exposure recently has been associated with the deficiency of Zn. Nutritional zinc deficiency can be caused from lead (Pb) exposure through dangerous cycle that increases lead absorption and increases Zn secretion consecutively [4]. Previous studies in animal experiments summarized that the conditions of marginal Zn deficiency, could increase blood lead levels in the presence of Pb exposure [5,6].

Among school children, chronic Pb exposure is correlated with nutritional deficiencies, anemia, impairments to physical growth, cognitive deficient and learning disorders [7 - 10]. Anemia is a major public health problem especially in developing countries. Additionally, WHO reported that of 25,4% anemia has been found in school children [11]. Anemia is a state of decreasing of erythrocytes and hemoglobin, whereas those condition impaired its function to carry oxygen through body tissues sufficiently [12,13]. Hemoglobin is composed of four globin chain polypeptide whereas in each chain contains a heme molecule containing Iron [14]. As well as iron, zinc also plays an important role in the formation of hemoglobin. In heme biosynthesis, δ-ALA dehydratase enzyme that catalyzes charge 2 δ-ALA into molecules that is highly dependent on zinc [15]. A study conducted in Atlanta gives us information that there is a significant correlation between serum zinc concentrations and hemoglobin levels since children who suffered iron deficiency anemia tend to be deficiency of zinc serum [16]. Normal zinc level has a consequence on erythrocytes life period, due to zinc contributes in protecting erythrocytes from oxidative stress and cell damage [17]. Increasing of blood lead levels could also interfered with erythropoiesis by inhibiting the synthesis of protoporphyrin and the absorption of iron (Fe) thus increasing the risk anemia. Furthermore, lead also affects the morphology of erythrocytes [18].

Nowadays, rapid growth of industrialization and its waste became environmental issues in coastal region of Indonesia. The industrial waste that was dumped in the coastal region induced the increasing level of lead pollutant in this area [19]. The coverage of environmental lead toxicant was quiet wide, including the exposure of fisheries products, public water source, and air pollution [20]. Lead exposure among children is an increasing problem globally, adversely affecting the quality of life among large numbers of children. The impact of lead exposure can affect all ages, whereas the children could absorbed more than 50 % of lead that enters the body, compared with adults who absorbed only 15-35 % [21]. The major vehicle of its exposure in the human body consecutively through the food consumption (65 %), the water consumption (20 %) or through the air (15 %) which were contaminated by lead [22]. In area with lead exposure, anemia and/or zinc deficiency might be occurred with or without clinical manifestation [20].

Lead exposure can not be seen as only a trivial environment and health problem. A study depicted even though given iron and zinc supplementation on lead-exposed children did not reduce blood lead levels [23]. Recently in Indonesia study about lead exposure among children mostly conducted in urban area, but not in coastal region. Especially among school children in Semarang city coastal region, the linking between blood lead levels with serum zinc level, anemia, and red blood cell indices has been not fully investigated.

2. Materials and Methods

2.1. Study population and site

A cross sectional study was conducted on an elementary school in Tambak lorok coastal region, Semarang, Central Java Indonesia. Tambak lorok is located less than 3 Kilometers from coastal java sea and near to harbor industrial region (Figure 1). About 80 subjects in this study were recruited using consecutive sampling method and met the inclusion criteria. During data collection, 8 subjects were dropped out due to did not agree to collect the blood. There were 72 children have completed data collection.

Inclusion criteria in this study: age between 8 to 12 years, and did not suffer from infectious illness (diarrhea, pneumonia, tuberculosis, and parasites) which were evidenced by looking at health records in primary health care. Demographic characteristics focused on parental educational level, parental occupation, source of water consumption, consumption of seafood products, age, and sex. Body weight was measured using calibrated digital body weight scale with accuracy 0.1 Kilogram. Body height was obtained using board stadiometer with accuracy 0.1 Centimeters. Median z score for weight for age, height for age, and BMI for age were performed to describe children nutritional status.

2.2. Biochemical collection and analysis

Biochemical data in this study consists of serum zinc level, red blood cells indices (Hb, Ht, mcv, mch, mchc), and blood lead level. About 10 mL venous blood has taken in the morning day before subjects have a breakfast using disposable plastic syringe and immediately transferred into three sterile-vacutainer. A potassium anticoagulant - EDTA (Ethylene Diamine Tetra Acetate) vacutainer was injected with 2 mL venous blood. This vacutainer was used to measure red blood cells (RBCs, in x 10^6 /mL), hemoglobin (Hb, in g/dL), hematocrit (Ht, in g/dL) mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH), and mean corpuscle hemoglobin concentration (MCHC). Blood indices were analyzed using automated Nihon Cohden Celltac E MEK-7222K hematology analyzer. Ferritin serum was analyzed using ELISA method.

The other two non-additives vacutainer were injected with 3 mL for serum zinc measurement and 5 mL for blood lead measurement. Approximately 3 mL whole blood in non-additive vacutainer was centrifuged, then the blood serum was prepared to zinc serum analysis. Substantially 5 mL whole blood was centrifuged and incorporated with APDC (Ammonium Pyrrolidine Dithiocarbamate) 2%. The incorporated solution was then re-centrifuged, allowed to stand for 5 minutes and mixed with 2 ml of butyl acetate. That mixed solution was centrifuged again until separation occurs. The reading of serum zinc level and blood lead level were operated using the Atomic Absorbent Spectrophotometry (AAS) at a wavelength of 213.9 nm for zinc serum and 283.3 nm for blood lead (Shimadzu AA6401F, Japan). Biochemical measurement was organized in the micronutrient and IDD Center laboratory, Diponegoro University, Indonesia.

Dietary assessment was conducted to determine fish intake among this population. The three-non consecutive days 24-hour food recall method was carried out to describe fish intake. In this study, local fish products have also been collected from this coastal area. Environmental assessment has been conducted on drinking water samples were collected using standard methods. Lead contents from fish products was measured using Spectrophotometry. Lead analysis of local fish products and drinking water samples were carried out in Center of Prevention on Industrial Pollution Laboratory, Ministry of Industrial, Republic Indonesia.

2.3. Statistical analysis

The SPSS for IBM version 19 and Microsoft excel software were performed for statistical analysis. All the study variables were tested for normality by the Kolmogorov-Smirnov test. The Mann Whitney test was operated to compare the blood lead levels and zinc serum levels between subjects with anemia and non anemia. The Correlation between blood lead level and serum zinc, hb, ht, RBC, mcv, mch, and ferritin were performed using Spearman rank's correlation analysis. Statistical significance was considered when p value < 0.05.

2.4. Ethics

The study protocol was approved by the Board of Medical Ethics on Faculty of Medicine Diponegoro University / Dr. Kariadi Hospital with no. 534/EC/FK-RSDK/2015. The participants, who were recruited in this study, clearly informed about the purpose of investigations and expected outcomes. Informed consent was obtained and signed by the parents of the subjects before the study began.

3. Results

3.1. Demographic characteristics of respondents

The coastal region in this study was taken place at Tambak Lorok area, the northern area of Semarang, Central Java. This area borders along with java sea. The main harbor of Central Java province has been located in this coastal area. Industrial area that was built in this region may contributed to environmental and health problems issue. Figure 1 depicts environmental situation in this coastal region.

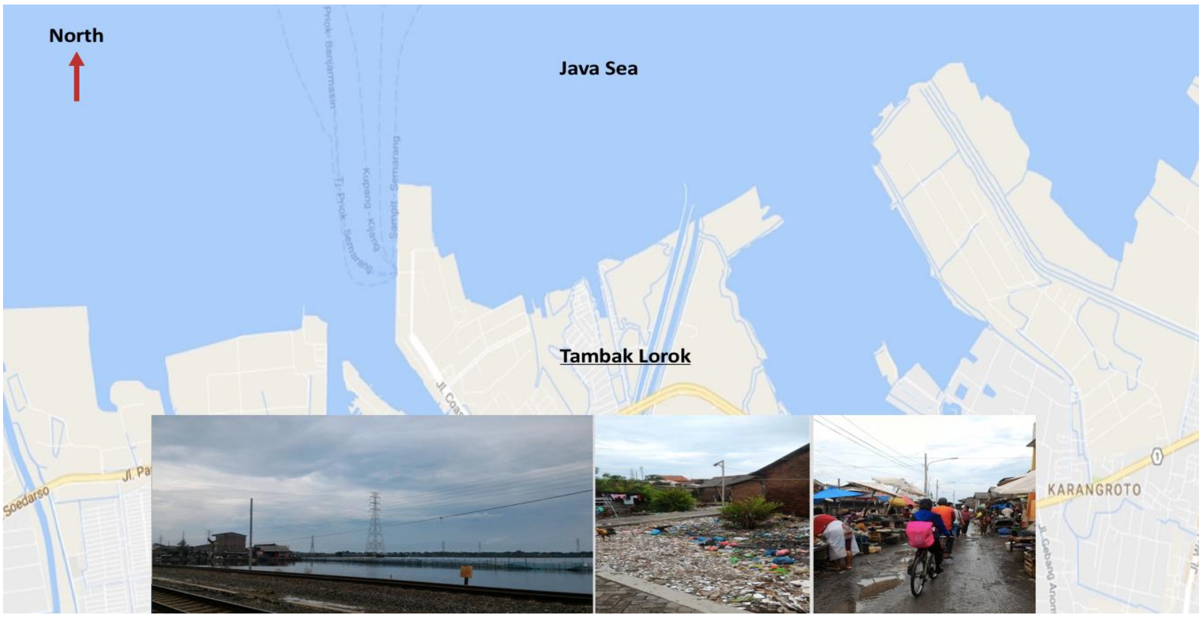

Figure 1. Environmental situation of Semarang coastal region (authors documented).

3.2. Biochemical data analysis

Table 1 shows values of haemoglobin, hematocrit, red blood indices, zinc serum levels, blood lead levels among all children. All subjects had blood lead levels $\geq$ 10 (µg/dl), low zinc serum levels (< 65 µg/dl), and median of ferritin describes < 15 ng/ml.

Table 1. Mean, SD, median, minimum and maximum values of blood indices, zinc serum, blood lead levels, and ferritin among subjects (N=72)

Biochemical parameters	Median	Range (min – max)
Haemoglobin (g/dl)	12.85	10,00 – 15,6
Hematocrit (g/dl)	35.40	28,70 – 44,70
RBC ($\times 10^6$ mm^3)	4.82	4.21 – 6.00
MCV(µ^3)	74.45	52.10 – 81.05
MCH	27.00	18.10 – 29.70
MCHC	36.30	34.10 – 38.30
Ferritin (ng/ml)	13.56	2.28 – 44.68
Zinc serum (µg/dl)	18.10	10,25 – 41,39
Blood Lead Level (µg/dl)	34.86	11.46 – 58.86

This study was carried out to 72 children with age range from 9 to13 years with a mean value of 10.16 ± 1.02 years. Approximately 66.7% of children's age were categorized between 9 and 10 years old.

Surprisingly, about 90.3% of children were stunting, consists of mild stunting 34.7%, moderate stunting 45.8%, and severe stunting 9.7%. We can see from table 1 about 26.4% children were anemic. Father's occupation of subjects largely as fisherman (59.7%). Both father and mother of children had literate, mostly graduated from Senior High School (father 51.4%, mother 45.8%) (Table 2).

Table 2. Characteristics of school age children (N=72)

Characteristics	N (%)
Sex	
Male	39 (54.2)
Female	33 (45.8)
Age	
9 – 10	48 (66.7)
11 – 12	24 (33.3)
Height for Age Nutritional Status	
Normal	7 (9.7)
Mild Stunting	25 (34.7)
Moderate Stunting	33 (45.8)
Severe Stunting	7 (9.7)
Anemia Status	
Anemic	19 (26.4)
Non Anemic	53 (73.6)
Father literacy	
Graduated elementary school	13 (18.1)
Graduated junior high school	22 (30.6)
Graduated senior high school	37 (51.4)
Mother literacy	
Graduated elementary school	16 (22.2)
Graduated junior high school	23 (31.9)
Graduated senior high school	33 (45.8)
Father's occupation	
Salesman	5 (6.9)
Fisherman	43 (59.7)
Labor	24 (33.3)
Mother's occupation	
Household	36 (50.0)
Salesman	2 (2.8)
Labor	34 (47.2)

Comparison between mean values of different hematological parameters, blood lead levels, zinc serum levels and serum ferritin in anemic and non anemic were figured out in this study (Table 3). Regarding the hematological parameters, nearly all values were significantly lower among the anemic than the non-anemic group except for blood lead levels, which showed a highly significant elevation among the anemic group. However, according to World Health Organization (WHO) cut off for blood lead levels, all children in this study had a blood lead ≥10 μg/dl (high blood lead level group {HBLLs}). As for the RBC count, ferritin, and zinc serum, no statistically significant difference was detected between the groups.

Table 3. Comparison between mean values of different hematological parameters, zinc serum, blood lead levels, and serum level of ferritin in anemic and non-anemic groups.

Biochemical parameters	Anemic Group (N=19)	Non Anemic Group (N=53)	Test of Significance
RBC ($\times 10^6$ mm^3)	4.82 $\pm$ 0.49	4.88 $\pm$ 0.33	t = - 0.56 p = 0.58
Hb (g/dl)	11.65 $\pm$ 0.62	13.26 $\pm$ 0.77*	t = - 9.03 p = 0.000
Hct	32.66 $\pm$ 1.44	36.47 $\pm$ 1.88*	t = - 9.05 p = 0.000
MCV(μ^3)	68.43 $\pm$ 7.50	74.89 $\pm$ 3.74*	t = - 4.84 p = 0.000
MCH	24.45 $\pm$ 3.05	27.26 $\pm$ 1.65**	t = - 5.01 p = 0.000
MCHC	35.66 $\pm$ 0.92	36.39 $\pm$ 0.75*	t = - 3.09 p = 0.005
Ferritin (ng/ml)	15.59 $\pm$ 8.39	15.36 $\pm$ 8.58	t = 0.10 p = 0.92
Zinc serum (µg/dl)	20.13 $\pm$ 9.64	20.44 $\pm$ 8.31	t = - 0.12 p = 0.90
Blood lead level (µg/dl)	41.95 $\pm$ 10.61	32.03 $\pm$ 10.33**	t = 3.52 p = 0.001

**independent t-test were performed (significant if p<0.05)*
***Mann Whitney test were performed (significant if p<0.05)*

Table 4 reveals the correlation between the different red blood indices, serum zinc levels and the blood levels of lead. Blood lead levels had a significant negative correlation with haemoglobin, hematocrit, and zinc serum ($r = -0.330$, $r = -0.328$, respectively $p<0.005$ and $r = -0.265$, $p < 0.05$).

Table 4. Correlation of different hematological parameters, serum iron and ferritin levels in relation to blood lead, copper and zinc.

Hematological parameters	Blood lead levels r-value (p-value)	Zinc serum levels r-value (p-value)
RBC ($\times 10^6$ mm^3)	-0.066 (0.585)	-0.128 (0.285)
Hb (g/dl)	-0.330 (0.005)	0.119 (0.319)
Hct	-0.328 (0.005)	0.101 (0.396)
MCV(μ^3)	-0.124 (0.298)	0.130 (0.276)
MCH	-0.109 (0.363)	0.148 (0.214)
MCHC	-0.106 (0.375)	0.144 (0.227)
Ferritin (ng/ml)	0.178 (0.135)	0.075 (0.529)
Zinc serum levels	-0.265 (0.025)	-
Blood lead levels	-	-0.265 (0.025)

3.3. Environmental assessment

Environmental assessment has been conducted on drinking water samples and dietary fish intake. Local fish products were collected based on the information from dietary fish intake interview. Lead content of drinking water and local fish products can be seen in Table 5.

Table 5. Fish intake (g per day) and lead levels of drink water and local fish products

Parameters	Mean	Median	Range (min – max)
Fish intake (g/day)		40	10,0 – 128
Lead from drinking water (µg/dl)		0	0
Lead from some type of fish (mg/Kg)			
- Green mussels (Pena viridis)	1.13		
- Scallop shell (Anadara granosa)	0.76		
- Kipper fish (Scatophagidae)	0.18		
- Snapper fish (Lates calcalifer)	0.13		
- Mullets fish (Mugil cephalus)	0.18		

4. Discussion

All school children in this study (100%) had BLL ≥ 10 µg/dl and zinc serum levels < 65 µg/dl, similar to a study done by Hegazy et al [7]. who also reported about more than a half subjects (63.3%) had BLL ≥ 10 µg/dl. However the current study is similar to the results obtained for children population in India [24]. The cut off value of l0 µg/dl defined by the WHO guideline for Childhood Lead Poisoning as a limit for an elevated blood lead level primarily is based on neurological toxicity and has wide range of toxicity including neurobehavioral impairment [21]. Recently, even though blood lead levels (BLL) less than 10 µg/dl is considered safe, a study confirmed that BLL < 10 µg/dl has associated with cognitive deficits [25]. Thus our data showed all children had low zinc serum levels (< 65 µg/dl). The Joint WHO/UNICEF/IAEA/IZiNCG asserted zinc serum levels < 65 µg/dl has been recognized as serious public health problems [26].

Schwartz et al [28] reported that children living near lead smelters in the US of Idaho, had blood lead levels approximately 25 µg/dl and were correlated with anaemia. In addition, Jain et al [29]. reported that children with BLL >10 µg/dl had 1.7 times risk to be moderate anaemia, in contrast with our finding showed that 73.6% of children had normal haemoglobin. Froom et al [30]. suggested that haemoglobin level did not correlate well with BLLs. However, our finding described that Hb, Hct, MCV, MCH and MCHC values of children with anaemia lower in comparison to anemic group. Moreover high BLLs has negatively associated with lower zinc serum and some blood indices levels. Lead absorption occurs predominantly in the duodenum and jejunum. The process of lead entry into the body may carry on through food, drink or by air. Approximately 90 % of lead absorbed by the blood binds to red blood cells [27]. The children absorbed up to 50% of lead from food and/or drink through the gastrointestinal tract and will be included in the metabolic processes of the body. Those lower red blood indices is similar with hypochromic microcytic anaemia which can be caused by blood lead toxicity [7]. Lead causes anaemia by impairing heme synthesis and increasing the rate of red blood cell destruction [31]. Although a causal pathway cannot be determined, yet the study findings clearly demonstrate the differences of BLLs between anaemia status.

In the present study zinc serum level of the anemic group is insignificantly different than non-anemic group. Lead (Pb) has the same valiancy number (Pb2+) as zinc (Zn2+) and iron (Fe2+), whereas on the cellular transport by Dimetal Transporter-1 (DMT-1) competition may be occurred [32]. Lead (Pb2+) will always be dominant to carry on by DMT-1 because of its density higher than Zn2+ and Fe2+

[27]. Lead (Pb) inhibits δ-ALA dehydratase enzyme that catalyzes δ-ALA into molecules, which is highly dependent to zinc on haemoglobin synthesis [15].

In the current study high BLLs among school children may be due to air pollution, sewage contamination, and probably from food consumption which were contaminated with Lead (Pb). Based on dietary interview, children consume fish regularly (range between 10 g/day and 128 g/day). Thus based on lead measurement content on some fish products showed variety lead content (mg/Kg fish products), green mussels (Pena viridis) had the highest content of lead (1.13mg/Kg). Green mussels (Pena viridis) has been vended on street food vendor near by school building. However a causal pathway between some fish products intake and blood lead levels cannot be firmed only from this data. A large epidemiology study on general northern coastal communities should remain a concern because of the nature of accumulation.

In developing countries such as Indonesia, control of lead contamination is much slower and the negative health effects getting more sporadic. The present work revealed that lead contamination should be considered as public health concern in northern coastal population of Semarang, Central Java, Indonesia.

5. Conclusion

In the present study, all subjects has high blood lead levels (BLLs) and low zinc serum. Blood lead levels (BLLs) were negatively associated with the hematological parameters and zinc serum levels. Lead (Pb) was not presented in drinking water, but Pb was discovered in some fish products that may be regularly consume by the children. Lead exposure could be controlled and strides should be taken to reduce zinc deficiency and anaemia among children population at coastal region.

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