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Reappraisal of Blood Lead Levels and Relation to Delta-Aminolevulinic Acid Dehydratase, Zinc Protoporphyrin and Urinary ALA Level in Thai Normal Adults and Lead Exposed Workers

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Abstract

All studied participants of 65 lead-exposed workers and 52 non lead-exposed persons who voluntarily participated and signed consents were interviewed about their working duration, type of work, risk behavior and personal data. Blood and urine samples were drawn for PbB, δ–ALAD, ZPP and ALAU analysis respectively. The mean PbB level for workers in lead smelters, 22.4 ± 17.9 µg/dl (range 1.3-67.2 µg/dl) was found higher than that for the non-exposed persons (mean = 5.0 ± 2.4 µg/dl, range 2.0-13.2 µg/dl). In relative to previous studies, mean PbB concentration found in the unexposed persons tended to decreased follows the reduced use of leaded gasoline in 1996 in Thailand. A highly significant negative correlation (R^2 = 0.86) was observed between PbB concentration and δ–ALAD activities among lead exposed persons. Statistical analysis showed that δ–ALAD activities significantly decreased ($p<0.05$) when PbB level surpassed 10 µg/dl. ZPP level increases slowly with increasing PbB concentration from 10-40 µg/dl and can be distinguished from normal when the PbB concentration surpasses 40 µg/dl. Whereas significant relationship between ALAU levels & PbB concentrations did not observed in lead exposed persons as well as non-lead exposed persons. The mean PbB level of 5.0 ± 2.4 µg/dl and range of 2.0-13.2 µg/dl could be regarded as the current reference values for the general population of the country. Determination of δ–ALAD activity in erythrocytes is a useful method for evaluating lead exposure in high blood lead level persons (PbB>10µg/dl). ZPP measurement is simple and inexpensive, but less sensitive and could be used for screening only in those high lead contaminated persons (PbB>40 µg/dl). ALAU measurement cannot be served as early biochemical indices of lead exposure both in lead exposed and non-lead exposed persons.

Keywords: bloodleadlevel; delta-aminolevulinic acid dehydratase; zinc protoporphyrin; urinary aminolevulinic acid

1. Introduction

Lead is a ubiquitous environmental pollutant, found in soil, water and air. Prolonged exposure to lead may cause a variety of adverse effects on the human body such as disturbance of the hematopoietic system, damage to the central or peripheral nervous system, chromosome aberration, decrease fertility, abnormal DNA formation and renal dysfunction (Seppalainen *et al.*, 1983). Markers of lead intoxication have been developed based on their capacity to identify lead intoxication at a preclinical stage. Delta-aminolevulinic acid dehydratase (δ–ALAD), a polymorphic enzyme that converts aminolevulinic acid to porphobilinogen in the heme biosynthetic pathway, is an effective biomarker of exposure to lead (Fleming *et al.*,1998). It is regarded as highly sensitive to inhibition from lead and it is an indicator of recent the removal of lead from gasoline in 1996. Traditional markers of lead intoxication, delta-aminolevulinic acid dehydratase (δ–ALAD), zinc protoporphyrin (ZPP) and urinary ALA (ALAU) were also measured and evaluated as preclinical markers.

2. Materials and methods

Blood and urine samples were obtained from 65 lead smelter workers in the mining area of Kanchanaburi province, western Thailand. Samples were also drawn from 52 non lead-exposed persons from outside the mining area. This general people was considered as a control group during the study. Blood samples were drawn by venipuncture using disposable heparinized syringes and placed in polypropylene tubes. Blood and urine samples were refrigerated at 4°C until the analysis of δ-ALAD, ZPP and ALAU activities respectively. The analysis was performed within two hours of sample collection. PbB was analyzed by Graphite furnace atomic absorption spectrophotometry using a Varian model SpectrAA 640Z and was expressed in µg/dl (Henry, 1979). The analytical process of blood lead was validated using certified reference material from Biorad laboratories, Germany, for which the coefficient of variation, and measurement accuracy were 5.25% and 95.5 ± 5% respectively. Blood δ–ALAD activity was measured following the method described by Burch and Siegel (1971) with the spectrophotometric determination carried out at 555 nm and expressed in unit/ml erythrocyte. ZPP was analyzed by hemofluorometry using a model 206 AVIV hemafluorometer and was expressed in mg/dl of whole blood (Zwennis *et al.*, 1990). ALAU activity was measured in urine following the method described by Makino *et al.*, (2000) with the spectrometric determination carried out at 553 nm and expressed in µg/dl.

All individuals were interviewed to collect information about their personal data and risk behavior. Statistical analyses were performed using SPSS statistical software. Differences of means among groups were tested by the Wilcoxon-Mann Whitney test. Spearman correlation was used to analyze the relationship between ALAD & PbB, ZPP & PbB and ALAU & PbB.

3. Results

3.1. Population Characteristics

Sixty-five lead smelter workers (61.5%, 40 males and 38.5%,25 females) participated in this study. Men's age ranged from 25 to 60 years (mean 31.5 years, and SD 10.2). Female participants were between 20 and 42 years of age (mean 32.2 years, SD 6.8). Average length of employment at the factory was 51 months. Only 4.6% of lead smelter workers smoked cigarettes. Fifty-two non-lead exposed persons (30 males and 22 females, age 20-58 years) were use as a control group.

3.2. PbB level and relationship to δ-ALAD, ZPP and ALAU activities

Mean PbB level for smelter workers, 22.4 ± 17.9 µg/dl (range 1.3-67.2 µg/dl) was higher than that for the non-exposed persons (mean = 5.0 ± 2.4 µg/dl, range 2.0-13.2 µg/dl) (Table1) as well as the current "admissible" limit of 10 µg/dl used in most countries. Almost 17% of the lead exposed workes have PbB level higher than 10 µg/dl whereas only 4 % of the non-exposed persons have PbB level higher than 10 µg/dl. Statistical analysis show that there was a significant difference ($p<0.05$) in PbB level by gender among lead exposed workers with males having the higher concentrations (Fig. 1). However, we did not observed a significant and expected relationship between PbB levels and tobacco consumption and working duration in both lead exposed and non-exposed groups. In contrast to lead exposed group, statistical analysis indicates that there was no significant difference ($p>0.05$) in PbB level by gender among non-exposed persons (Fig.1).

Mean δ- ALAD value in the lead exposed group was 198.1 ± 129.8 unit/ml erythrocyte (range 34.0 - 464.0 unit/ml erythrocyte) significantly lower than value in the non-exposed group (mean = 319.9 ± 78.3 range 188.0 - 554.0 unit/ml erythrocyte) (Table1).

In contrast mean ZPP level in the lead exposed group was found to be which is significantly higher ($p>0.05$) at 64.9 ± 38.9 µg/dl (range 30.0-220.0 µg/dl) than that in the non-lead exposed group, 51.5 ± 13.7 µg/dl (range 34.0-96.0 µg/dl) (Table1). Mean ALAU level in the lead exposed group, 0.8 ± 0.5 µg/dl (range 0-6.9 µg/dl) were not significantly different ($p>0.05$) from that in the non-lead exposed group, mean 1.4 ± 0.5 (range 0-7.3 µg/dl) (Table1).

A highly significant negative correlation ($R^2 = 0.86$) was observed between PbB concentration and δ-ALAD activities among lead exposed persons (Fig.2). The correlation equation was found to be δ-ALAD activities (unit/ml erythrocyte) = $362.58\ e^{-0.0383\ PbB\ (\mu g/dl)}$ ($n = 65$, $R^2 = 0.86$)

Statistical analysis showed that δ–ALAD activities significantly decreased ($p<0.05$) when PbB level surpassed 10 mg/dl. However, no correlation was observed between PbB concentration and δ-ALA-D activities among non-lead exposed persons. Since most of the participants (96%) in the non-

Table 1. Mean±S.D. and range of PbB, δ-ALAD, and ZPP in lead exposed (n=65) and non-lead exposed persons (n=52).

	PbB(µg/dl)		δ-ALAD (unit/ml erythrocyte)		ZPP (µg/dl whole blood)		ALAU(µg/dl)	
	lead exposed	non-lead exposed	lead exposed	non-lead exposed	lead exposed	non-lead exposed	lead exposed	non-lead exposed
Mean± S.D.	22.4±17.9	5.0±2.4	198.1±129.8	319.9±78.3	64.9±38.9	51.5±13.7	0.8±0.5	1.47±0.5
Range	1.3-67.2	2.0-13.2	34.0-464.0	188.0-554.0	30.0-220.0	34.0-96.0	0-6.9	0-7.3

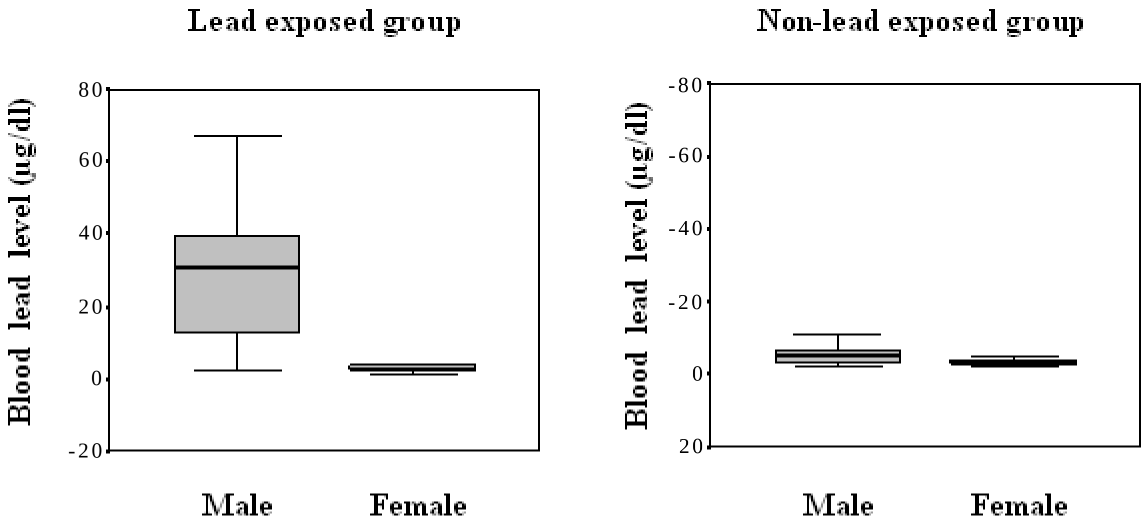

Figure 1. Blood lead levels among male and female in lead exposed and non-lead exposed persons.

lead exposed group have blood lead level lower than 10 mg/dl. A significant correlation ($R^2 = 0.64$) was observed between PbB concentration and ZPP level among lead exposed persons (Fig. 2). However, this study showed that ZPP level increases slowly with increasing PbB concentration from 10-40 µg/dl. ZPP level can be distinguished from normal when the PbB concentration surpasses 40 mg/dl. The correlation between PbB concentration and ZPP level was found to be *ZPP activities* (µg/dl whole blood)

$$= 35.661e^{0.0221\ PbB\ (\mu g/dl)} \quad (n = 65, R^2 = 0.64)$$

However, we did not observed a significant and expected relationship between ZPP level & PbB concentrations in non-lead exposed persons. Since none of the participants in non-lead exposed group has blood lead level over 40 µg/dl. In this investigation,we did not observed any significant relationships between ALAU levels & PbB concentrations in either groups.

4. Discussion

The mean PbB concentration found in the present study, 5 mg/dl for unexposed persons in Thailand is only a little over half of that reported for an equivalent group in 1993 (Ruangkanchanasetr and Suepiantham, 2002) and on which an admissible level of 10 mg/dl was set. This dramatic decline follows closely the reduced use of leaded gasoline that began in 1996 in Thailand (Table 2). Within four years mean PbB levels dropped to 5.58 mg/dl. More recently this level has continued to decrease albeit at a much slower rate to 5.00 mg/dl in 2007. With the benefit of this recent data and demonstrated trend of declining values, it may be time to reconsider the admissible for blood lead levels for the general population. Based on the results of our study, we proposed that the mean PbB level in the non-exposed persons of 5.0 mg/dl and range of 2.0-13.2 mg/dl could be regarded as the current reference values for the general population of the country.

Results of this study confirms with those of Pirkle *et al.*, (1998) who reported that blood lead levels among people in the general population have continued to decline in the United States to < 5 µg/dl due to introduction of lead-free gasoline. As a matter of fact that blood lead levels in the population depend on the ambient lead levels, a periodic surveillance survey and reappraisal of reference values is still recommended.

The mean PbB level in lead smelter workers, 22.4 µg/dl (range 1.3-67.2 µg/dl) was higher than that for the non-exposed persons (5.0 µg/dl). This mean level is almost similar level to those studies in the Bangkok traffic policemen in 1991(Yooyen *et al.,* 2000). The mean blood lead levels in traffic policemen were at 20.94, 24.81 µg/dl when the lead additives used in the leaded gasoline were at 0.4 grams/liter (Cheevaporn *et al.,* 2004). We considered these persons with mean PbB level of 22.4 µg/dl are the high risk group which a periodic surveillance survey to monitor blood lead level, is necessary.

Results of this investigation show that there was a significant difference ($p<0.05$) in PbB level by gender among lead exposed workers with males having the higher concentrations. The reason and biochemical basis that why male workers have higher lead level is not known. Therefore, we suggest that caution should be exercised in the sampling protocol of the high risk persons due to significant difference in PbB level by gender.

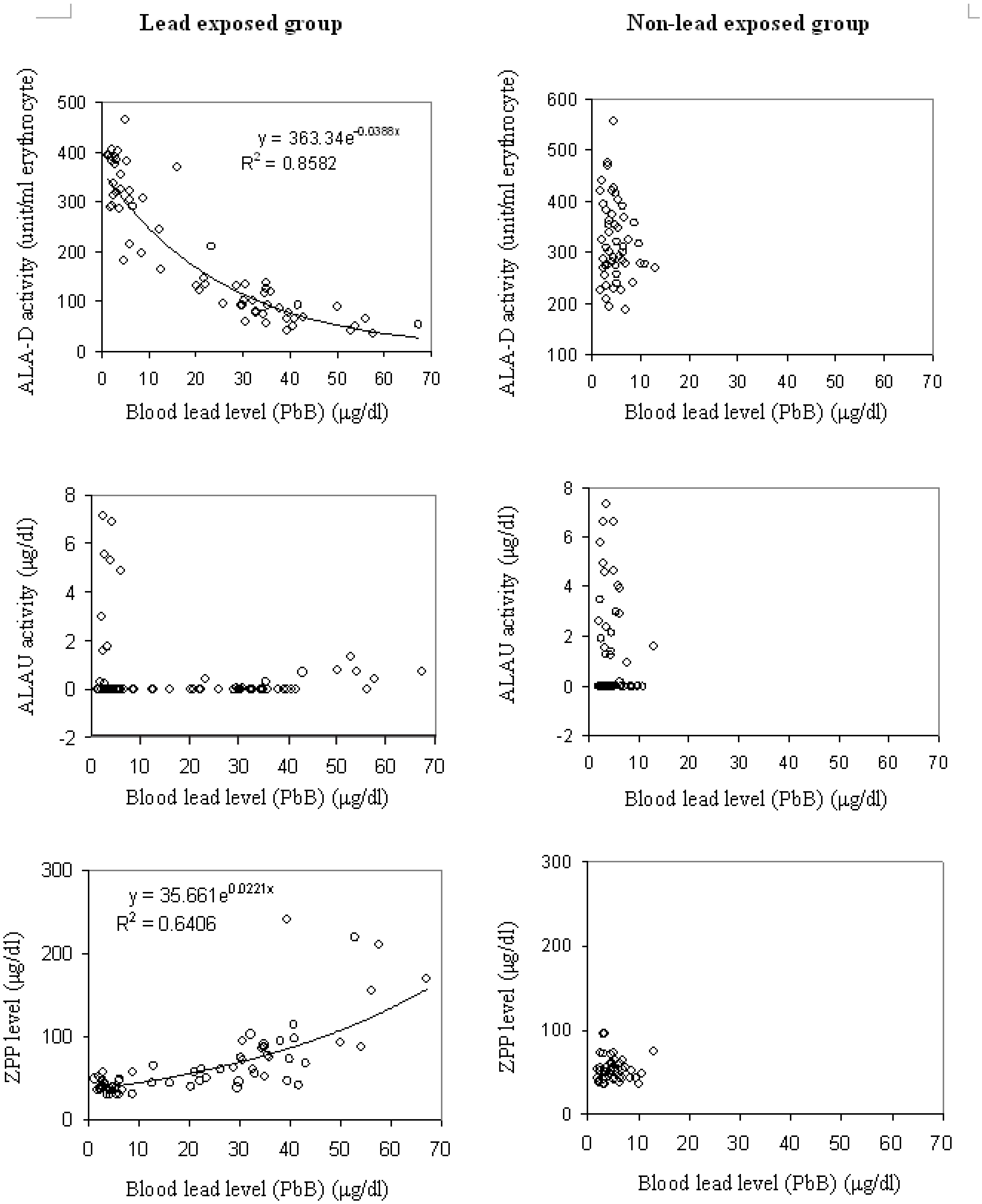

Figure 2. Correlation between ALAD activity, ALAU activity, ZPP level and blood lead level in lead exposed and non-lead exposed groups.

Table 2. Reduction of blood lead level in general populations before and after phase out of leaded gasoline in 1996.

Year	Mean blood lead level (μg/dl)	References
1993	9.23	(Ruangkanchanasetr and Suepiantham, 2002)
2000	5.58	(Ruangkanchanasetr and Suepiantham, 2002)
2007	5.00	this study

However, we did not observed a significant and expected relationship between PbB levels and tobacco consumption and working duration in both lead exposed and non-exposed groups. Similar results have been confirmed by Cardenas-Bustamante *et al.*, (2001) who studied the effects of lead on car battery workers in Bogota, Columbia.

Statistical analysis from this study reveals that δ-ALAD activities in the lead smelter workers are significantly lower in relative to the non-lead exposed group. Thus results of this study confirmed that the activities of these enzymes are severely inhibited by lead. Whereas the mean ZPP level in the lead exposed group were found significantly higher from that in the non-lead exposed group.This result indicated that lead obstructs the binding of iron to protoporphyrin IX, which in turn binds to zinc, forming zinc protoporphyrin (ATSDR, 1999).

A significant negative correlation was observed between PbB concentration and δ-ALAD activities among lead exposed persons. δ-ALAD activities significantly decrease when PbB level surpass 10 μg/dl. Results of this study confirmed that the measurement of δ-ALAD activities could be used as a sensitive biomarker for environmental lead exposure in the high risk person when lead blood concentrations surpass 10 μg/dl. However, measurement of δ-ALA-D activities is not a sensitive biomarker in the general population with low PbB level (<10 μg/dl). This result is in accord with the studies of Hernberg *et al.*(1970) and Nordman & Hernberg (1975) in lead exposed and selected western population in Scandinavia. Importantly, ALAU levels in the urine do not correlate closely with actual blood lead level in marked contrast to the findings reported in ATSDR (1999) and, based on the results of the present study, do not serve as an early indicator of lead exposure.

ZPP increased slowly with increasing PbB concentration in accord with observations reported by Landrigan (1990). However the increase is such that ZPP in lead exposed people cannot be distinguished from values in unexposed persons until PbB concentration surpasses 40 μg/dl. Thus, while it is a simple and inexpensive test, it is of relatively limited usefulness. In addition it should be noted that elevated ZPP levels in the absence of lead can be caused by iron deficiency anemia (Hyun-Cheol *et al.*, 1999) further restricting its usefulness in this regard.

In summary this study concluded that blood lead levels in the general population of Thailand has continued to decrease following the reduction of air lead levels due to the nation-wide gradual elimination of leaded gasoline. The mean PbB level in non-exposed persons of 5.0 μg/dl could be regarded as the current reference values for the general population of the country. There is a highly significant negative correlation between PbB concentration and δ-ALAD activity among lead exposed persons. Determination of δ-ALAD activity in erythrocytes is therefore a useful method for evaluating lead exposure in high blood lead level (>10μg/dl) persons. The activity is specifically inhibited when PbB concentration surpassed 10 μg/dl. ZPP does not serve as a useful indicator of lead exposure except at very high blood lead levels and this is complicated by its interaction with iron. Excretion of ALA in urine does not correlate

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