Lead Exposure and Accumulation in Healthy Thais: Assessed by Lead Levels, EDTA Mobilization and Heme Synthesis-related Parameters

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Abstract

Lead is one of the pollutants which is of public concern. The magnitude of lead contamination in Thai people is of interest. The objective of this study was to evaluate the lead status in normal healthy volunteers.

Normal volunteers were included. The blood for lead level, Zinc protoporphyrin (ZPP), δ-aminolevulinic acid dehydratase (ALA-D) activity, and baseline urine for lead, δ-aminolevulinic acid (ALA) and coproporphyrinogen III (CP_3) were collected. The EDTA mobilization test was done. 24 hour urine after administration of the drug was collected for lead analysis.

Thirty volunteers were included in the study. All were men whose average age was 32.5±6.9 years. The mean lead level was 5.95±2.01 μg/dL and 5.83±2.32 μg/L in urine. The 24 hour urine lead contents before and after EDTA administration were significantly different (11.11±6.72 and 16.05±9.51 μg respectively). Blood ALA-D activity was 251.6±80.4 unit/ml of RBC. Urine ALA and CP_3 were 0.56±1.2 mg/L and 22.17±23.9 μg/L respectively. All were in the normal ranges.

All parameters suggested that the healthy Thai volunteers had an acceptable magnitude of lead exposure and accumulation.

Lead is a non-biological metal necessary for human beings. Exposure to lead can cause acute and chronic toxicity. The aspects of lead related health effects have been raised and are of public concern. An increase in the lead level impairs the higher cortical function and contributes to chronic nephropathy(1-3). The principle source of contaminated lead is food. Others include water,

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air and dust in the environment[4]. Tetraethyl lead (TEL) in gasoline is another important source of lead to the environment.

Thailand has been changing to be an industrialized country. The effects of this are an increase of toxic waste and pollution. Bangkok, the capital of the country, is a rapidly growing city, overcrowded and suffering from a traffic problem. The health issues associated with the pollution in the city, especially lead are of concerns. The magnitude of the problem has been often raised.

Many studies regarding lead levels in Thai people have been reported[5-7]. Differences in methods and parameters to indicate the lead levels in the body may give different views. Blood lead level is used as a marker for indicating lead exposure[8-10]. However, it can not differentiate between acute exposure from chronic lead burden. Edetate calcium disodium (EDTA), a chelating agent for lead, can mobilize lead from the chelatable pool in the body and excreted in the urine. The amount of lead excreted in urine gives information to estimate the lead burden. The EDTA chelating (mobilizing or provocative) test has been considered as a surrogate for chelatable lead and lead burden[11,12]. Some investigators used heme synthesis products such as erythrocyte zinc protoporphyrin (ZPP), blood δ-aminolevulinic acid dehydratase (ALA-D) activity, urinary δ-aminolevulinic acid (ALA) and coproporphyrinogen III (CP_3) which are interfered by lead as markers for lead poisoning[13-15].

The objectives of this study were to establish the magnitude of lead exposure and accumulation in normal Thai people. Using available relevant parameters such as blood and urine lead levels, chelatable urine lead and heme synthesis related parameters to evaluate the lead status in Thai people.

MATERIAL AND METHOD

Subjects

Thai health adult volunteers whose occupations were not directly involved with lead took part in the study. The volunteers were interviewed, had a physical examination and laboratory tests. The tests included blood urea nitrogen, creatinine (BUN/Cr), urine analysis (UA) and liver function test. The eligible volunteers for the study should have no history of occupational lead exposure, normal physical examination, and the laboratory tests. These were done for excluding liver and renal diseases. The study was reviewed and approved by the Committee on Human Rights Related to Research Involving Humans, Faculty of Medicine, Ramathibodi Hospital, Mahidol University (No. 060/1996).

Drugs

Calcium Disodium Versenate® (edetate calcium disodium, EDTA) from Sanofi Wintrop, McPherson, Kansas, U.S.A.

Method

After the subjects were screened and had signed informed consents, they were told to continue with regular activities and diet. The day before the administration of EDTA, the subjects were instructed to collect 24 hour urine in acid washed containers. The specimens were collected for determining lead, ALA, CP_3 and creatinine concentration. On the study day, they emptied their bladders before administration of the drug. A heparinized catheter was inserted. One ml of blood was drawn in the acid washed heparinized tube for blood lead level, another 2 ml of blood in the EDTA tube for ZPP, ALA-D were collected. At time 0 (t=0), 1000 mg of edetate calcium disodium (EDTA) dissolved in 200 ml of 5 per cent dextrose in water was infused intravenously over 2 hours. Urine was collected from t=0 to t=24 in the acid washed container. Urine samples were analyzed for lead and creatinine. To make sure that the urine samples were completely collected, only urine samples with creatinine content of more than 80 per cent of their baseline were included for data analysis. The subjects were followed-up for 5 days after administration of the drug.

Blood and urine lead were analyzed by Varian Spectr AA-30/40 Zeeman graphite furnace atomic absorption spectrometer with programmable sample dispenser. The concentration of lead was determined by measurements of the curve peak area[16]. All results were reported as the mean of two separated determinations.

Blood for ALA-D was determined by Burch's method[17]. The Zinc protoporphyrin (ZPP) level was determined by Hematofluorometry[18]. The urine ALA and CP_3 were determined by Grabecki's and R. Askevold's methods respectively[19,20].

Statistics analysis included Student's *t*-test. P value of less than 0.05 was considered significant.

RESULTS

1. Subjects' characteristics:

Thirty one volunteers participated in the study, but data from 30 subjects were included in the study. One volunteer was excluded after finishing the study because his 24 hour urine collection after EDTA administration was not complete. All the subjects were male with an average age of 32.5±6.9 years (Table 1). They were workers, security guards, and office workers. All worked and lived in the Bangkok Metropolitan area.

2. Lead levels and the associated parameters shown in Table 2. The range of blood lead level was under 10 μg/dL which was in the acceptable limit[4,9]. There was no evidence of inhibition of ALA-D activity in our subjects. Urine ALA and CP_3 did not show the excess of their excretion.

Most subjects had normal urine CP_3 levels, ranging from 0-47 μg/L. One subject had the level of 135.96 μg/L, which was relatively higher than the others, but in the acceptable range. He had urine ALA of 0.0 mg/L, ALA-D activity 403 unit/ml of RBC, ZPP 19 μg/dL of RBC and blood lead 7.46 μg/dL, which were in the normal range. Another subject was anemic (Hct=24%), his ZPP increased slightly to 40 μg/dL of RBC. But other parameters were in the normal range (blood lead 4.53 μg/dL, ALA-D 149 unit/ml of RBC, ALA 0.0 mg/L, and CP_3 26.85 μg/L).

Table 3 shows the result of the EDTA mobilization test. Urine creatinine confirmed the adequacy of urine collection. The baseline amount of lead excreted in the urine was less than after EDTA administration ($p < 0.05$). However, both baseline and after EDTA administration was small in amount.

DISCUSSION

Blood lead concentration as a marker for lead exposure in our subjects was 5.95±2.01 μg/dL, comparable to other studies in many countries. A survey in Taiwan found an average blood lead of the Taiwanese population at 8.28±5.39 μg/dL[21]. A study in Korea revealed that the geometric mean was 6.36 μg/dL in men and 5.09 μg/dL in

Table 1 Characteristics of the subjects (n=30).

	Means ± S.D.	(Range)	
Age	32.5±6.9	(19-46)	year
Body weight	60.4±7.5	(50.5-80.0)	kg
Height	166.7±4.3	(160-175)	cm
Income	4,726±854	(4,100-8,000)	Baht/month

Table 2. Blood lead, urine lead levels and heme synthesis-related parameters of the subjects.

	Means ± S.D.	(Range)	Acceptable or reference range	
Lead levels				
Blood	5.95±2.01	(2.06-9.65)	< 40*	μg/dL
Urine	5.83±2.32	(0.96-10.15)		μg/L
ALA-D	251.6±80.4	(130-424)	132-374**	unit/ml of RBC
Urine ALA	0.6±1.2	(0.0-4.5)	0.0-6.0**	mg/L
Urine CP_3	22.2±23.9	(0.0-136.0)	0-200**	μg/L
ZPP	20.2±12.2	(2-40)	17-77**	μg/dL of RBC
Hct	41.4±5.3	(24-52)	40-54**	%

* The acceptable range

** The laboratory reference ranges.

Table 3. The 24 hour collected urine for creatinine and lead content before and after EDTA mobilization test.

	Baseline Mean±S.D. (Range)	After EDTA Mean±S.D. (Range)
Lead	11.11±6.72 (0.96-24.35)	16.05±9.51*** (4.08-41.41) μg/24 h
Creatinine	1,241±315	1,374±303 mg/24 h

*** There was significant difference between baseline and after EDTA urine lead (P=0.02).

women(22). The average blood level in women in Riyadh city, Saudi Arabia was 5.49±2.6 μg/dL(23). In Mexico city, the geometric mean of pregnant women was 6.7 and 11.1 μg/dL with respect to types of hospitals they attended(24). This level was slightly higher than that of people in the US, according to a study in pregnant women in Boston, Massachusetts. It showed a mean blood lead level of 6.7±3.91 μg/dL in 1976-1980 and declined to 1.19±1.31 μg/dL in 1990(25). The blood lead level in our study was acceptable. Although, our study was not a large scale survey in the general population, it might give a view of the Thai population, especially Bangkokians. Compared to previous studies in Thailand, the level was in the same magnitude found in a study of pregnant women in Ramathibodi Hospital in 1993. The mean lead level was 6.2±2.0 μg/dL(6). It was lower than a study in 1987, which found that people who lived near the main streets of Bangkok had an average blood lead of 15.6 μg/dL(5).

Tetraethyl lead (TEL) is an organic lead, added to petrol. It is another source of lead contamination to the environment. Since, organic lead has a different kinetic from inorganic lead(26,27). The blood lead level and total urine lead concentration might not be good indicators for inorganic lead intoxication(28). It is purposed that the urine lead concentration is a more reliable indicator for organic lead intoxication than blood lead(29). A study showed a urine concentration of 0-140 μg/L among normal salesmen and 200-1200 μg/L of TEL workmen with clinical evidence of poisoning(30). The concentration is rarely above 180 μg/L and the level of more than 350 μg/L suggested severe poisoning(29,31). The urine lead concentration in our study was low compared to the studies previously mentioned. (Table 2).

The enzyme δ-aminolevulinic acid dehydratase (ALA-D) is inhibited by lead during heme synthesis and results in an excess of blood δ-aminolevulinic acid (ALA). Coproporphyrinogen decarboxylase enzyme is also inhibited by lead, it causes coproporphyrinogen III (CP_3) accumulation. Both ALA and CP_3 are excreted in urine. They have been used as screening tests for lead poisoning(32,33). It was found that ALA-D inhibition is considered as the most sensitive indicator of lead interference and correlated with the blood lead of more than 10 μg/dL(13-15). Our findings showed normal ALA-D activity as well as normal levels of ALA and CP_3. These should confirm with the blood lead level to indicate that there was a low lead exposure in the subjects.

The EDTA provocative test is a useful test to estimate the lead body burden. A ratio of 24 hour urinary lead excretion (μg)/EDTA (mg) is determined. If the ratio is more than 0.7-1, it indicates a high lead store in the deep compartments and suggests a high risk for chronic lead poisoning(34-36). Our study showed that there was a statistical increase in urinary lead excretion after administering EDTA. But the absolute amount and the ratio of lead were small. This should suggest that the body burden of lead was minimal. Regarding zinc protoporphyrin (ZPP), lead blocks ferrochelatase enzyme from incorporating iron into protoporphyrin to form heme. The excess protoporphyrin binds with zinc and accumulates in the red blood cells. Increasing ZPP suggested chronic lead interference and correlated with blood levels of more than 30 μg/dL[37]. The normal level of ZPP in our subjects should be interpreted as a low lead burden.

Taking all the biological indicators together, our findings suggest that lead exposure and accumulation in our volunteers was low and in the acceptable level.

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การประเมินระดับและการสะสมของสารตะกั่วในคนไทยสุขภาพสมบูรณ์

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ตะกั่วเป็นสารพิษสำคัญที่ปนเปื้อนในสิ่งแวดล้อม ปัญหาการปนเปื้อนของตะกั่วในคนไทยโดยเฉพาะกรุงเทพ-มหานครเป็นภาวะที่ต้องเฝ้าระวัง คณะได้ทำการศึกษาหาระดับและสภาวะของตะกั่วในคนปกติที่อาศัยและทำงานที่ไม่เกี่ยวข้องกับตะกั่ว อยู่ในกรุงเทพฯและปริมณฑล โดยการใช้การตรวจหาค่าชีวภาพหลายชนิดที่เกี่ยวข้องกับตะกั่ว ซึ่งเป็นตัวบ่งชี้ถึงระดับและปริมาณตะกั่วในร่างกาย เพื่อใช้ประกอบเป็นเกณฑ์ในการประเมินระดับตะกั่วในร่างกายและสภาวะตะกั่วเป็นพิษ จากอาสาสมัคร 30 คนอายุเฉลี่ย 32.5±6.9 ปี พบว่าค่าเฉลี่ยระดับตะกั่วเท่ากับ 5.95±2.01 ไมโครกรัม/เดซิลิตรในเลือด และ 5.83±2.32 ไมโครกรัม/ลิตรในปัสสาวะ ปริมาณตะกั่วในปัสสาวะเท่ากับ 11.11±6.72 ไมโครกรัม/24 ชั่วโมง และเพิ่มเป็น 16.05±9.51 ไมโครกรัม/24 ชั่วโมง หลังจากได้ยา edetate disodium 1000 มิลลิกรัม ฉีดเข้าเส้นเลือด ระดับเอนไซม์ δ-aminolevulinic acid dehydratase (ALA-D) เท่ากับ 251±80 หน่วย/มิลลิลิตรของเม็ดเลือดแดง ความเข้มข้นของสาร δ-aminolevulinic acid (ALA) เท่ากับ 0.6±1.2 มิลลิกรัม/ลิตร, coproporphyrinogen III (CP_3) เท่ากับ 22.2±23.9 ไมโครกรัม/ลิตร และ zinc protoporphyrin (ZPP) 20.2±12.2 ไมโครกรัม/เดซิลิตรของเม็ดเลือดแดง ซึ่งค่าทั้งหมดอยู่ในเกณฑ์ปกติ การศึกษานี้แสดงว่าระดับของตะกั่วในร่างกายอยู่ในเกณฑ์ปลอดภัย และไม่พบว่ามีตัวบ่งชี้ใดที่แสดงถึงภาวะเป็นพิษจากตะกั่วในอาสาสมัคร

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