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ORIGINAL ARTICLE

Impact of Chronic Lead Exposure on Selected Biological Markers

Ambica P. Jangid · P. J. John · D. Yadav · Sandhya Mishra · Praveen Sharma

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Abstract Lead poisoning remains a major problem in India due to the lack of awareness of its ill effects among the clinical community. Blood lead, δ-aminolevulinic acid dehydratase (δ-ALAD) and zinc protoporphyrin (ZPP) concentrations are widely used as biomarkers for lead toxicity The present study was designed to determine the impact of chronic lead exposure on selected biological markers. A total of 250 subjects, of both sexes, ranging in age from 20 to 70 years, were recruited. On the basis of BLLs, the subjects were categorized into four groups: Group A (BLL: 0–10 μg/dl), Group B (BLL: 10–20 μg/dl). Group C (BLL: 20–30 μg/dl) and Group D (BLL: 30–40 μg/dl) having BLLs of 3.60 ± 2.71 μg/dl, 15.21 ± 2.65 μg/dl, 26.82 ± 2.53 μg/dl and 36.38 ± 2.83 μg/dl, respectively. Significant changes in biological markers due to elevated BLLs were noted. The relation of BLL and biological markers to demographic characteristics such as sex, habits, diet and substances abuse (smoking effect) were also studied in the present investigation. Males, urban population, non-vegetarians, and smokers had higher blood lead levels. δ-ALAD activity was found to be significantly lower with increased BLL ($P < 0.001$), while the ZPP level was significantly higher with increased BLL ($P < 0.001$). Further, BLL showed a negative correlation with δ-ALAD ($r = -0.425$, $P < 0.001$, $N = 250$) and a positive correlations with ZPP ($r = 0.669$, $P < 0.001$, $N = 250$). Chronic lead exposure affects the prooxidant-antioxidant equilibrium leading to cellular oxidative stress.

Keywords Blood lead level · δ-amino levulinic acid dehydratase · Zinc protoporphyrin

A. P. Jangid · P. J. John (✉)
Environmental Toxicology Laboratory, Department of Zoology, University of Rajasthan, Jaipur 302004, Rajasthan, India
e-mail: placheriljohn@yahoo.com

D. Yadav · S. Mishra
Department of Biochemistry, S.M.S. Medical College, Jaipur 302004, India

P. Sharma
Department of Biochemistry, Government Medical College, Kota 240009, Rajasthan, India

Introduction

Lead (Pb) is a normal constituent of the earth's crust, with trace amounts found naturally in soils, plants and water. If left undisturbed, lead (Pb) is practically immobile. However, once mined and transformed into man-made products, it is distributed throughout the environment, and becomes highly toxic. Solely as a result of man's action, lead (Pb) has become the most widely scattered toxic metal in the world. A growing body of evidence indicates that transition metals act as a catalyst in the oxidative deterioration of biological macromolecules. Therefore, the toxicities associated with these metals may lead to oxidative tissue damage. Several studies identified various biomarkers of lead (Pb) toxicity. Lead is a divalent cation. It has a strong binding capacity for sulfahydryl proteins and creates an interference with enzymes and structural proteins. Lead inhibits heme biosynthesis and causes anemia, basophilic stippling, a decrease in erythrocyte δ-aminolevulinic acid dehydrogenase and increase in urinary in δ-aminolevulinic acid, urinary coproporphyrin, erythrocyte zinc protoporphyrin (ZPP), and pyrimidine 5′-nucleotidase [1]. A marked increase in urinary excretion of aminolevulinic acid (ALA), the substrate that accumulates as a result of decreased ALAD, has been

used in the past as a biomarker for lead (Pb) toxicity. This can be detected only when BLLs exceed 35 μg/dl in adults and 25–75 μg/dl in children [2]. The most well-known distortions involved is the interference of lead with the heme synthetic pathway, specifically the enzyme δ-aminolevulinic acid dehydratase. Interference with heme production and subsequent reduction of the heme body pool is one of the main causes of lead related pathology. When whole blood lead levels (BLLs) exceed 20 μg/dl, the activity of ALAD is inhibited by 50 percent [3]. ALAD can serve as a valuable biomarker of oxidative stress in the lead exposed hematological system. It is also a biochemical indicator of lead exposure [4].

Thus the present study was undertaken to examine the blood lead levels and to assess the impact of chronic lead exposure on δ-ALAD and ZPP in the blood of normal healthy subjects.

Materials and Methods

Selection of Subjects

The study sample consisted of 250 normal healthy subjects. These subjects belonged to the age group of 20–70 years and included both male and female individuals. They were randomly selected from the urban and rural populations of Jaipur.

A written consent was obtained from all 250 subjects. Questionnaires for the subjects were completed during face-to-face interviews. All subjects answered the same questions regarding demographic data, socioeconomic status, habits, perceived health, and health complaints. The subjects suffering from any major disease (malignancy, hypertension, diabetes mellitus, arthritis, tuberculosis, heart disease, endocrine disorders etc.) that affects oxidative stress were excluded from the study.

Collection of Blood Sample

Blood samples from each subject was collected from the antecubital vein using aseptic techniques at Central Laboratory, Department of Biochemistry, SMS, Medical College, Jaipur. Each sample consisted of 5 ml of blood which was collected in a dipotassium ethylenediaminetetraacetic acid (EDTA) vial and the following investigations were performed on this sample.

1. Blood lead levels (BLLs)
2. Delta-aminolevulinic acid dehydratase (δ-ALAD)
3. Zinc protoporphyrin (ZPP)

Biochemical Assay

Blood Lead (Pb)

The blood samples collected from the subjects were analyzed for lead (Pb) level by using Atomic absorption spectrophotometer (AAS). Sample pre-treatment consisted of a fivefold dilution with a dilute surfactant. The instrument was directly calibrated with lead standards prepared in dilute HNO_3. To eliminate small, nonspecific absorption signals from the blood matrix, simultaneous background correction was used. The lead was accurately measured from as little as 20 μl of blood at wavelength of 283.3 nm [5].

Delta-Amino Levulinic Acid Dehydratase (δ-Alad)

Delta-aminolevulinic acid (δ-ALAD) was measured spectrophotometrically. The enzyme delta-amino levulinic acid dehydratase converts two molecules of ALA to porphobilinogen. The porphobilinogen formed was mixed with modified Ehrlich's reagent and the color development was measured at 555 nm [6].

Zinc Protoporphyrin (Zpp)

Zinc protoporphyrine (ZPP) was detected by a hematofluorometer. A small (unmeasured) drop of blood was obtained from a finger puncture. This was then placed on a disposable cover slip and inserted into the sample holder of the instrument. The zinc protoporphyrin concentration was automatically and instantaneously computed and the value was displayed on a digital readout as micrograms of zinc protoporphyrin per liter of blood at 424 nm [7].

Statistical Analysis

An appropriate statistical analysis was done using statistical software SPSS 10. Tukey's test was used for one way analysis of variance (ANOVA) in the current investigation to establish the significance in various groups. F-test was done to establish that the differences in the results of the present study were significant. Correlation was done by Pearson's correlation.

Results

All the 250 subjects selected in the present study were further divided into four groups that is group-A, group-B, group-C and group-D depending upon the blood lead (Pb) concentration. The subjects belonging to group-A ($N = 103, 41.00\%$)

had BLLs between 0 and 10 μg/dl and this group was considered to be the safest as per CDC and WHO guidelines [8, 9]. In group-B ($N = 67$, 27.00%), the lead level was 10–20 μg/dl. In group-C ($N = 53$, 21.00%), the lead level was 20–30 μg/dl and in group-D ($N = 27$, 11.00%), the lead level was 30–40 μg/dl (Table 1; Fig. 1). The BLLs of group-B, C and D were compared to the BLL of group-A.

Table 2 shows the alterations in BLLs and biological markers in chronic lead exposure with reference to demographic characteristics such as sex, habitat, diet and substance abuse (smoking) of selected subjects. A significantly lower BLL and a significantly higher δ-ALAD and ZPP were observed in females as compared to males. In urban and non-vegetarian subjects, the BLL and ZPP were found to be significantly increased and the δ-ALAD was found to be significantly decreased as compared to rural and vegetarian subjects. A significantly lower BLL and ZPP and significantly higher δ-ALAD were found in non-smokers as compared to smokers.

Table 3 shows the alteration in δ-ALAD and ZPP levels in subjects of various BLL groups. The decrease in δ-ALAD level was statistically significant in group-C and D but not significant in group-B as compared to the safest group-A. However, the increase in levels of ZPP is statistically significant in group-B, C and D when compared to the safest group-A.

Table 4 shows the correlation between blood lead levels and the levels of the biomarkers of lead toxicity using Pearson's correlation. A significant negative correlation of blood lead level was observed with the δ-ALAD ($r = -0.425$, Fig. 2) and a positive correlation of blood lead levels was found with ZPP ($r = 0.669$, Fig. 3).

Discussion

Lead as such is a metal which has no known function in body and any amount of it may be associated with adverse effects. More recently WHO has reduced the cut off of BLLs and said that the level should be less than 10 μg/dl because any amount of lead in blood causes adverse effects at the cellular level. There are certain studies which have

Table 1 Blood lead (Pb) level range groups according to concentration of lead

Groups	Blood lead level range	Mean ± SD
Group-A (103)	0–10 μg/dl	3.60 ± 2.71
Group-B (67)	10–20 μg/dl	15.21 ± 2.65
Group-C (53)	20–30 μg/dl	26.82 ± 2.53
Group-D (27)	30–40 μg/dl	36.38 ± 2.83

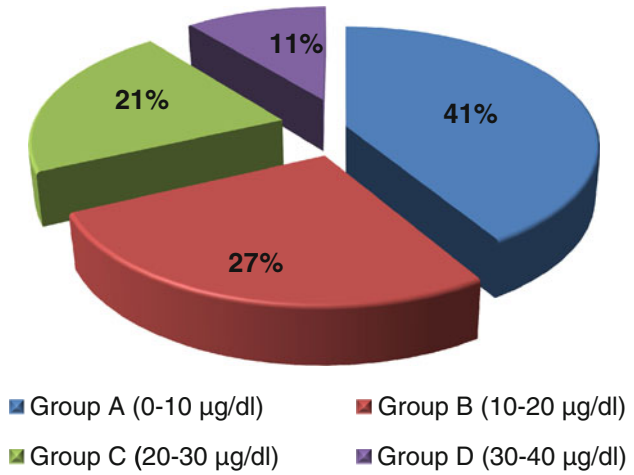

Fig. 1 Distribution of subjects according to blood lead concentration

related blood lead concentration above 10 μg/dl in children and adolescent with various neurological dysfunctions [10]. In the present study out of 250 participants, only 40% subjects had blood lead level below 10 μg/dl. The remaining 60% subjects had blood lead concentration of more than 10 μg/dl of these 11% had BLL above 30 μg/dl (Table 1; Fig. 1). BLL has been considered as one of the most reliable markers for lead toxicity [4].

Out of 250 participants, 217 subjects were males and 33 were females and 141 were from rural background and 109 were from urban background. 153 were vegetarians while 97 were non-vegetarians and 139 subjects were smokers and 111 were non-smokers.

The BLLs were found to be significantly higher in males as compared to females ($P < 0.05$). This can be attributed to the fact that males are relatively more exposed to pollution in a variety of occupations as compared to females [11]. When δ-ALAD value in both the genders were examined the level was lower in males as compared to females and the difference was significant ($P < 0.05$). Variability of ZPP value in male and female subjects was found to be significantly ($P < 0.05$) higher in males as compared to female subjects. This has been supported by several other researchers [12, 13].

In urban subjects the BLL was found to be significantly higher as compared to subjects from the rural background. The value being between 23.23 ± 9.80 and 8.93 ± 7.18 (Table 2). Elevated lead (Pb) concentration in blood in urban population can be ascribed to increased exposure to various sources of lead (Pb) which includes pollution through paints [14], lead recycling, presence of lead in cosmetics [15, 16] and in drinking public water supply in lead pipes [17, 18]. The mean δ-ALAD value was found to be 40.53 ± 15.10 and 34.47 ± 17.28 U/l in rural and urban subjects, respectively. There was a significant

Table 2 Alterations in biological markers in chronic lead exposure with reference to demographic characteristics

Subjects	Selected biological markers		
	Lead (μg/dl)	Delta-ALAD(U/l)	ZPP (μg/l)
Male ($N = 217$)	15.55 ± 11.88	37.71 ± 16.31	39.05 ± 16.67
Female ($N = 33$)	11.56 ± 10.86*	39.05 ± 16.67*	42.57 ± 25.98*
Rural ($N = 141$)	8.93 ± 7.18	40.53 ± 15.10	37.46 ± 16.81
Urban ($N = 109$)	23.23 ± 9.80*	34.47 ± 17.28*	55.28 ± 28.31*
Vegetarian ($N = 153$)	12.65 ± 10.34	39.28 ± 15.99	40.79 ± 18.98
Non-vegetarian ($N = 97$)	19.13 ± 12.91*	35.70 ± 16.70*	52.30 ± 29.41*
Smokers ($N = 139$)	17.16 ± 12.92	35.88 ± 17.32	47.42 ± 26.17
Non-smokers ($N = 111$)	11.56 ± 10.86*	39.05 ± 16.67*	44.58 ± 25.98*

* Significant ($P < 0.05$, F test)

Table 3 Influence of selected biological markers in subjects of various BLL groups

Parameters	BLL groups				ANOVA (single factor)
	A(0–10 μg/dl) ($N = 103$)	B(10–20 μg/dl) ($N = 67$)	C(20–30 μg/dl) ($N = 53$)	D(30–40 μg/dl) ($N = 27$)	
δ-ALAD(U/l) 95% CI (mean)	41.81 ± 14.43 (38.99–44.63)	42.75 ± 14.16^{NS} (39.29–46.20)	33.41 ± 16.68* (28.81–38.01)	19.67 ± 12.84* (14.59–24.75)	$F = 20.31$, $P < 0.001$
ZPP (μg/l) 95% CI (mean)	30.83 ± 10.54 (10.74–152.33)	44.16 ± 17.60* (39.87–48.46)	54.45 ± 15.61* (50.14–58.75)	84.20 ± 36.27* (69.85–98.54)	$F = 70.21$, $P < 0.001$

Values represent mean ± standard deviation and 95% CI for mean

Mean values with '*' are significantly different from safest group-A, whereas value with 'NS' are not significant according to Tukey's test

Statistical analysis was done using ANOVA single factor

Table 4 Correlation between BLL and selected biological markers ($N = 250$)

Correlation	δ-ALAD (U/l)	ZPP (μg/l)
Lead (μg/dl)	$r = -0.425$, $P < 0.001$	$r = 0.669$, $P < 0.001$

r Correlation coefficient (Pearson's correlation)

decrease in the concentration of δ-ALAD in urban population as compared to rural population ($P < 0.05$). In urban subjects the ZPP level was relatively higher as compared to rural subjects ($P < 0.05$).

The BLL was found to be significantly increased in non-vegetarians as compared to vegetarians. This may due to the fact that the non-vegetarians are mostly found to be in the urban setup and to have a greater chance to get exposed to lead pollution as compared to vegetarians. Current investigations reveal significant modulations in the level of δ-ALAD in vegetarian and non-vegetarian subjects ($P < 0.05$). The mean δ-ALAD value was found to be 39.28 ± 15.99 and 35.70 ± 16.7 U/l in vegetarian and non-vegetarian, respectively. Non-vegetarian and urban populations are associated with increased BLL. The decrease in δ-ALAD activity may be due to the binding of lead (Pb) with –SH groups of ALAD leading to its depressed activity as observed by various workers [4, 19, 20]. The level of ZPP in non-vegetarian subjects was also recorded to be higher as compared to the levels of this enzyme in vegetarian.

The BLL of smokers were significantly higher than lead levels in non-smokers ($P < 0.05$). This may be attributed to the fact that smokers are relatively more exposed to pollution in a variety of occupations as compared to non-smokers. When δ-ALAD value in both the smokers-non smokers were examined the level was lower in smokers as compared to non smokers and the difference was significant ($P < 0.05$). Variability of ZPP value in smokers-non smokers subjects were found to be significantly ($P < 0.05$) higher in smokers as compared to non-smokers subjects [21].

Table 3 shows the alterations in δ-ALAD and ZPP in subjects of various BLL groups. Reduced δ-ALAD concentration was found with elevation in BLLs in subjects which is evident from the coefficient of correlation value of $r = -0.425$ (Table 4; Fig. 2). The obtained results showed a significant difference in δ-ALAD of different groups ($P < 0.001$). Other studies have also shown similar results [4, 19, 20, 22]. Lead binds to the enzymes that have functional sulfhydryl groups, rendering them nonfunctional and further contributing to the impairment of oxidative

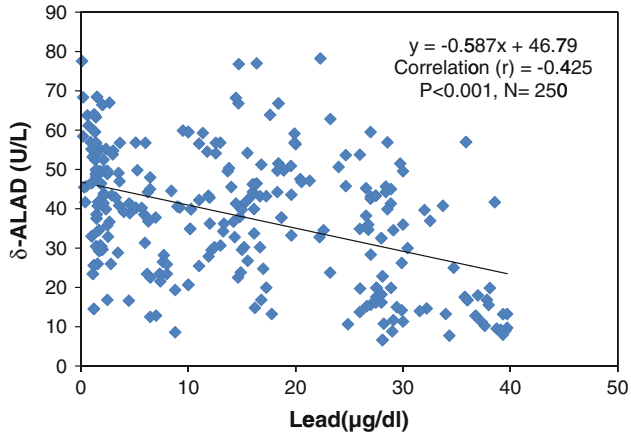

Fig. 2 Correlation between lead and δ-ALAD

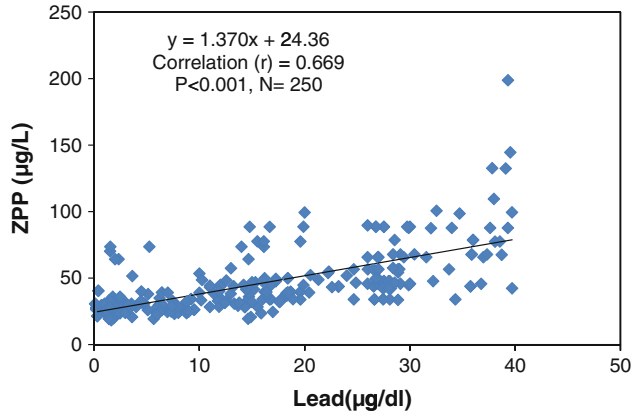

Fig. 3 Correlation between lead and ZPP

balance. Levels of two specific sulfhydryl containing enzymes that are inhibited by lead δ-ALAD and glutathione reductase (GR) have been demonstrated to be depressed in both animal and human lead exposure studies [4, 23–25]. Delta-ALAD is one of the enzymes of heme biosynthesis which converts ALA to porphobilinogen, the first precursor of pyrrole. Inhibition of δ-ALAD prevents ALA from being converted to porphobilinogen, inhibiting incorporation of iron into the protoporphyrin ring, resulted in impaired heme synthesis for hemoglobin. Decreased activity of δ-ALAD results in impaired heme biosynthesis [23]. It has also been reported that blood lead (Pb) concentration above 20 µg/dl inhibit activity of δ-ALAD by 50% which could lead to lead (Pb) associated metabolic abnormalities. Subjects with lead levels above the safe limit were found to have reduced δ-ALAD activity. The correlation between blood lead concentration and δ-ALAD was found to be negative with the correlation coefficient $(r) = -0.425$ but this is not linear (Fig. 2). Though the δ-ALAD indicates lead (Pb) toxicity but it cannot be taken as a biomarker for lead toxicity. Similar observations have been put forward by Phillip and Gerson [3]. Austrin et al. observed a 50% inhibition of δ-ALAD activity at BLL of 15 µg/dl [26]. Sakai and Morita reported that the threshold of extremely low blood lead for δ-ALAD inhibition was approximately 5 µg/dl [27].

ALAD is a crucial enzyme in lead toxicity. The inhibition of ALAD lowers heme production and increases the levels of the substrate delta-aminolevulinic acid (ALA). Elevated levels of ALA, found both in the blood and urine of subjects with lead exposure, are known to stimulate ROS production [28]. However, the heme precursors accumulate in erythroblasts and inhibit the ALAD [29]. ALAD was suggested to be too sensitive to lead (Pb) inhibition which makes it ineffective as an index of lead exposure [30]. The literature indicates that blood ALAD levels correlates very closely with blood lead levels, and serve as an early biochemical index of exposure which can also detect the lower levels of exposure [31].

Studies of lead workers have shown that δ-ALAD activity, correlates inversely with BLL [4]. General population studies indicate that the activity of ALAD is inhibited at very low BLLs, with no threshold yet apparent. Studies of children from India and China have reported the significant decreases in ALAD activity are associated with BLLs ≥10 µg/dl. Further the inverse correlations between BLL and ALAD activity were found in mothers (at delivery) and their newborns (cord blood) [32].

Blood lead levels and ZPP activity are used as clinical indices of lead toxicity [4]. In the present study the ZPP level was found to be significantly higher in subjects with elevated BLL and has been found to have a positive association (Table 3; Fig. 3, $P < 0.001$). There was a positive correlation between ZPP and BLLs which is evident from the coefficient of correlation value of $r = 0.669$ (Table 4; Fig. 3), which points to the inhibition of heme synthesis by ZPP. An increase in ZPP activity is because lead is known to inhibit the activity of ferrochetalase which catalyses the last step of the heme synthesis. Here lead is incorporated into protoporphyrin instead of iron, resulting in the production and accumulation of zinc protoporphyrin [33]. Similar findings were reported in other studies [4, 14, 22]. However, in the abundance of haemoglobin, increased ZPP is relatively harmless [34]. Elevated ZPP is more pronounced with blood Lead concentration above 30 µg/dl. This is because the threshold for ZPP in adult is 30 and 15 µg/dl in children. The findings of the present study also confirm the above phenomenon. Ferrochelatase enzyme that catalyzes the insertion of iron into protoporphyrin IX is also impaired by lead. Interruption of this enzyme results in an increase of the substrate erythrocyte protoporphyrin (EP), when bound to iron, zinc and zinc protoporphyrin (ZPP). These elevations do not appear in the blood until lead levels reach 35 µg/dl [35]. However, most protoporphyrin in erythrocytes (about

90%) exists as ZPP. Elevated erythrocyte protoporphyrin can reflect iron deficiency, sickle cell anemia and jaundice [35]. Other diseases such as porphyria, liver cirrhosis, iron deficiency, age and alcoholism may also produce similar effects on heme synthesis [2].

Conclusions

Lead poisoning is an old but persistent public health problem throughout world. Although guidelines for the management of lead poisoning were released by Centers for Disease Control and World Health Organization, no suitable approach for the treatment of low level lead poisoning appears to exist. Lead is an important environmental toxicant in developed and developing nations. It may cause serious health problems. When the antioxidant free radical scavenging systems are overwhelmed, pathological condition may occur. The results of the present study will help to establish the relationship between blood lead levels and the biological markers at the cellular level. BLL determinations remain the most suitable method for monitoring recent lead toxicity, whereas increased ZPP concentrations, even with concurrent nontoxic BLL, have a predictive value for detecting incipient lead toxicity. However, ALAD was found to be unsuitable as an index of lead exposure.

References

1. Sakai T. Reviews on biochemical markers of lead exposure with special emphasis on heme and nucleotide metabolism. Sangyo Eiseigaku Zasshi. 1995;40:314–7.
2. Somashekaraiah BV, Venkaiah B, Prasad AR. Biochemical diagnosis of occupational exposure to lead toxicity. Bull Environ Contam Toxicol. 1990;44:268–75.
3. Phillip AT, Gerson B. Lead poisoning-Part I. Incidence, etiology, and toxicokinetics. Clin Lab Med. 1994;14:423–44.
4. Gurer-Orhan H, Sabir HU, Ozgunes H. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. Toxicology. 2004;195:147–54.
5. Fernandez FJ. Micromethod for lead determination in whole blood by atomic absorption, with use of the graphite furnace. Clin Chem. 1975;21:558–61.
6. Wigfield DC, Farrant JP. Assay of delta-aminolaevulinate dehydratase in 10 microlitre blood. Clin Chem. 1981;27(1):100–3.
7. Blumberg WE, Esinger J, Lamola AA, Zuckerman DM. The haematofluorometer. Clin Chem. 1977;23:270–1.
8. CDC (Centers for Disease Control and Prevention). Preventing lead poisoning in young children. Public Health Service, Centers for Disease Control, US Department of Health and Human Services, Atlanta, Georgia. 1991. http://www.cdc.gov/nch/lead/publicaion. Accessed 1 Jan 2008.
9. WHO (World Health Organization). International Programme on Chemical Safety (IPCS). Inorganic lead. Environmental health criteria 165. Geneva. 1995. http://www.inchem.org/documents/ehc/ehc/ehc165.htm. Accessed 21 Apr 2008.
10. Baghurst PA, McMichael AJ, Wigg NR, Vimpani GV, Robertson EF, Roberts RJ, Tong SL. Environmental exposure to lead and children's intelligence at the age of seven years. New Engl J Med. 1992;327:1279–84.
11. Muntner P, Menke A, DeSalvo KB, Rabito FA, Batuman V. Continued decline in blood lead levels among adults in the United States: the National Health and Nutrition Examination Surveys. Arch Intern Med. 2005;165:2155–61.
12. Roels HA, Lauwerys RR, Buchet JP, Vrelust MT. Response of free erythrocyte porphyrin and urinary-delta-aminolevulinic acid in men and women moderately exposed to lead. Int Arch Arbeitsmed. 1975;34:97–108.
13. Grandjean P, Lintrup J. Erythrocyte–Zn–protoporphyrin as an indicator of lead exposure. Scand J Clin Lab Invest. 1978;38:669–75.
14. Mohammad IK, Mahdi AA, Raviraja A, Najmul I, Iqbal A, Thuppil V. Oxidative stress in painters exposed to low lead levels. Arh Hig Rada Toksikol. 2008;59:161–8.
15. Sprinkle RV. Leaded eye cosmetics: a cultural cause of elevated lead levels in children. J Fam Pract. 1995;40(4):358–62.
16. Sharma DC, Seervi N, Rawtani J. Effect of environmental lead pollution on hemoglobin and erythrocyte ALAD activity. Indian J Physiol Pharmacol. 2000;44(1):117–8.
17. Quinn MJ, Sherlock JC. The correspondence between U.K. 'action levels' for lead in blood and in water. Food Addit Contam. 1990;7:387–424.
18. WHO (World Health Organization). Lead in drinking water. Background document of WHO guidelines for drinking water quality, Geneva, WHO (WHO/SDE/WSH/03.04/09). 2003.
19. Ahamed M, Verma S, Kumar A, Siddiqui MK. Delta-aminolevulinic acid dehydratase inhibition and oxidative stress in relation to blood lead among urban adolescents. Hum Exp Toxicol. 2006;25(9):547–53.
20. Ahamed M, Singh S, Behari JR, Kumar A, Siddiqui MK. Interaction of lead with some essential trace metals in the blood of anemic children from Lucknow. India Clin Chim Acta. 2007;377(1–2):92–7.
21. Hulea SA, Olinescu R, Nita S, Crocnan D, Kummerow FA. Cigarette smoking causes biochemical changes in blood that are suggestive of oxidative stress: a case-control study. J Environ Pathol Toxicol Oncol. 1995;14(3–4):173–80.
22. Flora SJS, Singh S, Tandon SK. Plumbism among Indian silver jewellery industry workers. J Environ. Sci Health Part A 1990;25A (2):105–113.
23. Farant JP, Wigfeld DC. Biomonitoring lead exposure with delta-aminolevulinate dehydratase (ALA-D) activity ratios. Int Arch Occup Environ Health. 1982;51:15–24.
24. Sandhir R, Julka D, Gill KD. Lipoperoxidative damage on lead exposure in rat brain and its implications on membrane bound enzymes. Pharmacol Toxicol. 1994;74:66–71.
25. Ahamed M, Verma S, Kumar A, Siddiqui MK. Environmental exposure to lead and its correlation with biochemical indices in children. Sci Total Environ. 2005;346(1–3):48–55.
26. Austrin KH, Bishap DF, Wetmur JG, Kaul BC, Davidow B, Desnick RJ. Aminolevulinic acid dehydratase isozymes and lead toxicity. Ann NY Acad Sci. 1987;514:23–9.
27. Sakai T, Morita Y. Delta-aminolevulinic acid in plasma or whole blood as a sensitive indicator of lead effects, and its relation to the other heme-related parameters. Int Arch Occup Environ Health. 1996;68:126–32.
28. Bechara EJ. Oxidative stress in acute intermittent porphyria and lead poisoning may be triggered by 5-aminolevulinic acid. Braz J Med Biol Res. 1996;29:841–51.
29. Sakai T. Biomarkers of lead exposers. Ind Health. 2000;38:127–42.

30. Chiba M, Shinohara A, Matsushits K, Watanabe H, Inaba Y. Indices of lead exposure in blood, urine of lead exposed workers, concentrations of major, trace elements, activities of SOD, GsH-Px and catalase in their blood. Tohoku J Exp Med. 1996;178: 49–62.
31. Goyer H, Clarkson TW. Toxic effects of metals. In: Klassen CD, editor. Casarett and Doull's toxicology. The basic science of poisons. New York: McGraw-Hill; 2001. p. 830.
32. Jin Y, Liao Y, Lu C. Health effects in children aged 3–6 years induced by environmental lead exposure. Ecotoxicol Environ Saf. 2006;63(2):313–7.
33. Marcus AH, Schwartz J. Dose-response curves for erythrocyte porphyrin vs. blood lead, effects of iron status. Environ Res. 1987;44:221–7.
34. Onalaja VO, Claudio L. Genetic susceptibility to lead poisoning. Environ Health Perspect. 2000;108:23–8.
35. ATSDR (Agency for Toxic Substances and Disease Registry). Toxicological profile for lead. (Draft for Public Comment). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. 2007;232. http://www.atsdr.cdc.gov/ToxProfile/tp.asp. Accessed 1 Jan 2008.