Evaluation of Low Blood Lead Levels and Its Association with Oxidative Stress in Pregnant Anemic Women: A Comparative Prospective Study

Amit Kumar Mani Tiwari · Abbas Ali Mahdi ·
Fatima Zahra · Sudarshna Sharma ·
Mahendra Pal Singh Negi

Received: 24 November 2011 / Accepted: 3 March 2012 / Published online: 29 March 2012
© Association of Clinical Biochemists of India 2012

Abstract To correlate blood lead levels (BLLs) and oxidative stress parameters in pregnant anemic women. A total of 175 pregnant women were found suitable and included for this study. Following WHO criteria, 50 each were identified as non-anemic, mild anemic and moderate anemic and 25 were severe anemic. The age of all study subjects ranged from 24–41 years. At admission, BLLs and oxidative stress parameters were estimated as per standard protocols and subjected with ANOVA, Pearson correlation analysis and cluster analysis. Results showed significantly ($p < 0.01$) high BLLs, zinc protoporphyrin (ZPP), oxidized glutathione (GSSG), lipid peroxide (LPO) levels while low delta aminolevulinic acid dehydratase (δ -ALAD), iron (Fe), selenium (Se), zinc (Zn), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell (RBC) count, reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and total antioxidant capacity

(TAC) in all groups of anemic pregnant women as compared with non anemic pregnant women. In all groups of pregnant women, BLLs showed significant ($p < 0.01$) and direct association with ZPP, GSSG and LPO while inverse relation with δ -ALAD, Fe, Se, Zn, Hb, Hct, MCV, MCH, MCHC, RBC, GSH, SOD, CAT and TAC. Study concluded that low BLLs perturb oxidant-antioxidant balance and negatively affected hematological parameters which may eventually Pb to Fe deficiency anemia during pregnancy.

Keywords Iron deficiency anemia · Pregnancy · δ -ALAD · ZPP · Oxidative stress · Cluster analysis

Introduction

Over the last two decades there has been a significant worldwide increase in awareness and concern about the adverse effect of lead (Pb) on human health and the environment. Pb is one of the most abundant heavy metal present in the earth's crust and widely distributed and mobilized in the environment [1]. Pb is related to a broad range of physiologic, biochemical and behavioral dysfunctions [2]. There are reports that Pb initiates oxidative damage to heart, kidney, reproductive organs, brain and erythrocytes [3]. Moreover, Pb is known to have toxic effects on membrane structure and functions. It has been reported that erythrocyte membranes are more susceptible to Pb mediated damage as erythrocytes have high affinity for this metal [4, 5]. Elevated Pb levels have been associated with anemia, decreased IQ, impaired attention and speech performance and hypertension [6].

Although Pb toxicity in children and adults is well recognized, exposure to Pb is of special concern during

A. K. M. Tiwari · A. A. Mahdi (✉)
National Referral Centre For Lead Poisoning, UP,
Department of Biochemistry, C.S.M. Medical University,
Lucknow 226003, India
e-mail: mahdiaa@rediffmail.com

F. Zahra
Department of Obstetric & Gynecology, ELMC & Hospital,
Lucknow, India

S. Sharma
Department of Biochemistry, Bundelkhand University,
Jhansi, India

M. P. S. Negi
Institute for Data Computing and Training, Jankipuram,
Lucknow, India

pregnancy. Pb absorbed by the pregnant mother is readily transferred to the developing fetus [7, 8]. There is evidence from animal studies that intrauterine exposure to Pb may disrupt endocrine balance during pregnancy [9, 10], and lead to abnormalities of renal structure and function [11], abnormalities of the reproductive system [12], and neurodevelopmental toxicity [13] in offspring. Human evidence corroborates these findings, linking prenatal exposure to Pb with reduced birth weight and preterm delivery [14] and with neurodevelopmental abnormalities in offspring [15, 16]. These concerns are especially salient for women and children in developing nations. Not only is exposure to Pb common, but the toxicity of Pb for pregnant women and their offspring may be amplified by nutritional deficiency [17, 18] and concomitant toxic exposures [19] which often occur in poor nations.

There have been reports that nutrition plays an important role in Pb toxicity process. Nutrient factors, such as calcium, iron (Fe), zinc (Zn), phosphorous and proteins and personal factors including sex, age and genetic susceptibility can modify Pb toxicity. Nutrient interactions with Pb have provided evidence that deficiency of nutrients enhances Pb absorption and its toxicity [20]. As Fe is an essential element and plays a critical role in the heme synthetic pathway, therefore, the effect of Pb toxicity on Fe metabolism has been explored for several decades. It has been reported that more Pb is absorbed from the gastrointestinal tract and it causes more toxic effects in case of Fe deficient animals as well as human subjects [21]. The discovery of Fe binding protein in human duodenal mucosa, which competitively binds to Pb, facilitates Pb–Fe interaction research [22].

Most previous studies have focused on the relationship between dietary Fe intake and environmental Pb-exposure. Few investigators have examined plasma level of Fe in anemic women exposed to Pb. In this study, we estimated plasma Pb levels in the Fe deficient pregnant anemic women and assessed Pb induced oxidative stress and its adverse effects on antioxidant defense system.

Materials and Methods

Subjects

At admission, we estimated blood lead level (BLL) and blood profiles of 324 pregnant women attending outdoor patient department in the Department of Obstetrics and Gynaecology, Queen Mary's Hospital, Chhatrapati Shahuji Maharaj Medical University, Lucknow, U.P., India. Of 324, we randomly selected BLL and blood profiles of 239 pregnant women. As per our inclusion and exclusion criteria, a total of 175 were found suitable and included for

this study after obtaining the Institutional ethical approval and informed consent from them. Following WHO criteria for anemic and non-anemic, 50 were identified as non-anemic (haemoglobin, Hb > 11.0 g/dl), 50 each with mild (Hb: 10.0–10.9 g/dl) and moderate (Hb: 7.0–9.9 g/dl) anemic and 25 were severe (Hb < 7.0 g/dl) anemic [23]. The age of all study subjects ranged from 24 to 41 years. Care was taken to ensure that all the study subjects belonged to same middle socioeconomic class (who were able to meet the basic necessities of life and same requirements of comfort), with similar food habit and not taking any drugs preceding 1 month of the admission. We excluded women having Hb less than 6.5 g/dl, as these women may require immediate blood transfusion. All included pregnant women were non-alcoholic, non-smoker, and normotensive. Pregnant women having a history of metabolic diseases such as diabetes mellitus, malignancy, and heart disease, infections such as tuberculosis, HIV, endocrine disorders and women who have been using minerals and/or vitamin supplements were also excluded from the study.

Sample Collection

At admission, 5 ml venous blood was taken from each subject and divided into two aliquots at the time of recruitment. 3 ml blood was transferred to a heparin containing evacuated tube and used to determine Hb, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red blood cells (RBCs) count, glutathione (GSH), glutathione disulphide (GSSG), Pb, zinc protoporphyrin (ZPP) and delta aminolevulinic acid dehydratase (δ -ALAD). Another 2 ml of whole blood was transferred into heparin containing tube and then centrifuged, plasma separated and used for the estimation of lipid peroxide (LPO), Fe, Zn, and selenium (Se), while the remaining RBCs was lysed by mixing chilled water and RBC lysate was used for the estimation of catalase (CAT) and superoxide dismutase (SOD).

Analytical Estimation

Blood Hb was determined by using the cyanomethemoglobin method [24]. Haematocrit (Hct), MCV, MCH, MCHC and RBC counts were determined by using Sysmax A-380 automated cell counter. The estimation of Fe, Zn, Se and Pb on flame atomic absorption spectrophotometer (AAS) using a direct method as described by Kaneko [25]. The method is based on the property of atoms emitting element-specific electromagnetic radiations under assay conditions absorbed the energy (light) at that particular wavelength has been measured. The instrument was

calibrated using aqueous standards of various Pb concentrations (10–40 µg). Blood δ -ALAD activity was measured as per European standardized method [26]. ZPP levels were directly measured in whole blood by hematofluorometer [27]. For the determination of GSH and GSSG we used 5,5'-dithio-bis-2 nitrobenzoic acid (DTNB) as described by Ellman [28]. The LPO levels were measured by the method of Okhawa et al. [29]. The thiobarbituric acid reacting substances (TBARS) of the sample were estimated spectrophotometrically at 532 nm and expressed as nmol of MDA/mg protein. CAT (EC 1.11.1.6) activity was assayed as per the method of Aebi [30]. The CAT activity was expressed as mmol H₂O₂ catabolized/min/mg protein. The SOD (EC 1:15.1.1) activity was determined from its ability to inhibit the reduction of NBT in presence of PMS according to the method of Mc Cord and Fridovich [31]. The reaction was monitored spectrophotometrically at 560 nm. The SOD activity was expressed as U/mg protein (1 unit is the amount of enzyme that inhibit the reduction of NBT by one half in above reaction mixture). Total protein of sample was determined by the method of Lowry et al. [32]. Total antioxidant capacity (TAC) was estimated by ferric reducing ability of plasma (FRAP Assay) where antioxidant power converts ferric to ferrous ion reduction at low pH causing a colored ferrous tripyridylfrazine complex [33].

Statistical Analysis

Groups were compared by one way analysis of variance (ANOVA) followed by Newman–Keuls post hoc test. Pearson correlation analysis was used to evaluate association among variables. Similarity between variables and groups was done by cluster analysis (Hierarchical clustering; Single linkage and Euclidean distances) after standardizing the data, i.e., each variable has mean 0 and standard deviation of 1. A two tailed ($\alpha = 2$) probability $p < 0.05$ was considered to be statistically significant.

Results

Table 1 summarizes BLL, markers of Pb toxicity (δ -ALAD and ZPP) and plasma essential trace elements (Fe, Se & Zn) of four groups. The BLL levels were significantly ($p < 0.01$) higher in all anemic groups as compared to control group. In contrast, the δ -ALAD activity in moderate ($p < 0.05$) and severe ($p < 0.01$) anemic group lowered significantly as compared to control group. However, ZPP levels were higher significantly ($p < 0.01$) in all anemic groups as compared to control group. Moreover, the plasma trace elements especially Fe and Zn lowered significantly ($p < 0.05$ or $p < 0.01$) in all anemic groups as compared to control group.

Table 2 summarizes the haematological parameters (blood index values) of four groups. All haematological parameters lowered significantly ($p < 0.05$ or $p < 0.01$) in all anemic groups as compared to control group except MCHC. The blood index values of MCHC did not differed ($p > 0.05$) in mild anemic group and control group. Similar decrement pattern of haematological parameters were found in all anemic groups. The severe anemic group had shown the highest degree of decrement followed by moderate and mild the least (severe > moderate > mild).

Table 3 summarizes the oxidant (LPO) and antioxidant (CAT, SOD, TAC, GSH and GSSG) parameters of four groups. In all anemic groups, the levels of GSSG and LPO were significantly ($p < 0.05$ or $p < 0.01$) higher while levels of GSH, SOD, CAT and TAC were significantly ($p < 0.05$ or $p < 0.01$) lower as compared to control group.

Figures 1 and 2 summarize the similarity among variables and groups respectively of all pregnant women. The variables of all pregnant women clustered in two groups. The BLLs, ZPP, GSSG and LPO formed the first cluster of similar characteristics while δ -ALAD, Fe, Hb, TAC, Hct, MCH, SOD, MCV, CAT, GSH, Se, RBC, MCHC, and Zn formed the another cluster of similar characteristics. Similarly, the variables of all pregnant women grouped in two

Table 1 BLLs, markers of Pb toxicity and plasma trace elements in healthy and anemic women

Parameters	Control ($n = 50$)	Mild ($n = 50$)	Moderate ($n = 50$)	Severe ($n = 25$)
BLL (µg/dl)	1.84 ± 0.12	1.98 ± 0.13	2.61 ± 0.11 ^{ab}	3.62 ± 0.17 ^{abc}
ZPP (mg/g Hb)	2.44 ± 0.13	8.18 ± 0.37 ^a	11.58 ± 0.49 ^{ab}	16.32 ± 0.81 ^{abc}
ALAD (U/l)	15.14 ± 0.76	14.29 ± 0.43	12.79 ± 0.54 ^a	9.16 ± 0.69 ^{abc}
Fe (mg/dl)	51.46 ± 1.31	33.96 ± 1.21 ^a	24.13 ± 0.82 ^{ab}	17.62 ± 0.73 ^{abc}
Se (µmol/l)	1.71 ± 0.038	1.61 ± 0.034	1.49 ± 0.025 ^{ab}	1.33 ± 0.033 ^{abc}
Zn (µmol/l)	10.23 ± 0.16	9.57 ± 0.17 ^a	9.17 ± 0.17 ^a	8.63 ± 0.28 ^{ab}

Values are expressed in mean ± SE. Characters in superscript 'a', 'b' and 'c' represents 'control', 'mild', and 'moderate' groups respectively and were significantly different with respective group either at $p < 0.05$ (normal font) and $p < 0.01$ (italic font)

BLL blood lead level, ZPP zinc protoporphyrin, ALAD δ -aminolevulinic acid dehydratase

Table 2 Blood index values (Mean $\pm$ SE) of healthy and anemic women

Parameters	Control ($n = 50$)	Mild ($n = 50$)	Moderate ($n = 50$)	Severe ($n = 25$)
Hb (g/dl)	12.84 $\pm$ 0.17	10.29 $\pm$ 0.05 ^a	8.13 $\pm$ 0.11 ^{ab}	6.61 $\pm$ 0.14 ^{abc}
Hct (%)	34.83 $\pm$ 0.54	30.49 $\pm$ 0.49 ^a	25.98 $\pm$ 0.55 ^{ab}	21.98 $\pm$ 1.06 ^{abc}
MCV (fl)	84.12 $\pm$ 1.13	74.26 $\pm$ 0.86 ^a	67.36 $\pm$ 1.41 ^{ab}	62.39 $\pm$ 1.76 ^{abc}
MCH (pg)	30.16 $\pm$ 0.39	28.48 $\pm$ 0.47 ^a	25.55 $\pm$ 0.56 ^{ab}	20.71 $\pm$ 0.29 ^{abc}
MCHC (g/dl)	32.23 $\pm$ 0.28	31.12 $\pm$ 0.44	30.52 $\pm$ 0.47 ^a	26.93 $\pm$ 0.53 ^{abc}
RBC ($\times 10^{12}/l$)	5.18 $\pm$ 0.12	4.69 $\pm$ 0.16 ^a	3.95 $\pm$ 0.15 ^{ab}	3.79 $\pm$ 0.22 ^{ab}

Values are expressed in mean $\pm$ SE. Characters in superscript 'a', 'b' and 'c' represents 'control', 'mild', and 'moderate' groups respectively and were significantly different with respective group either at $p < 0.05$ (normal font) and $p < 0.01$ (italic font)

Hb haemoglobin, *Hct* haematocrit, *MCV* mean corpuscular volume, *MCH* mean corpuscular haemoglobin, *MCHC* mean corpuscular haemoglobin concentration, *RBC* red blood cell

Table 3 Oxidant and antioxidant parameters (Mean $\pm$ SE) of healthy and anemic women

Parameters	Control ($n = 50$)	Mild ($n = 50$)	Moderate ($n = 50$)	Severe ($n = 25$)
GSH (μ M)	413.81 $\pm$ 5.72	365.97 $\pm$ 6.61 ^a	341.13 $\pm$ 7.19 ^{ab}	331.02 $\pm$ 5.12 ^{ab}
GSSG (μ M)	145.95 $\pm$ 2.93	173.29 $\pm$ 3.39 ^a	191.36 $\pm$ 4.14 ^{ab}	216.23 $\pm$ 4.15 ^{abc}
LPO (U/mg protein)	2.45 $\pm$ 0.07	2.89 $\pm$ 0.13 ^a	4.11 $\pm$ 0.15 ^{ab}	5.27 $\pm$ 0.24 ^{abc}
SOD (U/mg protein)	1.15 $\pm$ 0.02	0.97 $\pm$ 0.02 ^a	0.86 $\pm$ 0.03 ^{ab}	0.68 $\pm$ 0.02 ^{abc}
CAT (U/mg protein)	56.57 $\pm$ 1.81	44.39 $\pm$ 1.82 ^a	36.66 $\pm$ 1.71 ^{ab}	28.19 $\pm$ 1.37 ^{abc}
TAC (μ mol/l)	3.95 $\pm$ 0.61	2.88 $\pm$ 0.091 ^a	2.47 $\pm$ 0.074 ^{ab}	1.78 $\pm$ 0.076 ^{abc}

Values are expressed in mean $\pm$ SE. Characters in superscript 'a', 'b' and 'c' represents 'control', 'mild', and 'moderate' groups respectively and were significantly different with respective group either at $p < 0.05$ (normal font) and $p < 0.01$ (italic font)

GSH reduced glutathione, *GSSG* oxidized glutathione, *LPO* lipid peroxide levels, *SOD* superoxide dismutase, *CAT* catalase, *TAC* total antioxidant capacity

clusters. The control and mild formed the first cluster of similar characteristics while moderate and severe formed another cluster of similar characteristics. In other words, in all four groups, variables induces oxidative stress in the order of severe > moderate > mild > control.

Discussion

We observed significant alterations in oxidant and antioxidant parameters of anemic pregnant women when compared with controls and this showed that oxidative damage can occur even at low BLLs. A strong correlation between blood Pb concentration and oxidative stress markers such as CAT, SOD and lipid peroxidation products was observed suggesting a possible contribution of Pb induced oxidative damage in anemic patients.

We also observed considerable reduction and a significant negative correlation of δ -ALAD with blood Pb (Fig. 1), following a significant increase and a positive correlation of ZPP with BLLs of anemic women compared to controls (Table 1), suggesting inhibition of heme synthesis even at low BLLs. Our study is in accordance with the earlier reports of Austrin et al. [34] who found 50 %

inhibition of δ -ALAD activity at a BLL of 15 μ g/dl and that of Sakai and Morita [35] who reported that threshold value of blood Pb for δ -ALAD inhibition was extremely low (approximately 5 μ g/dl). Our study showed an increase in ZPP levels because Pb is known to inhibit the activity of the enzyme ferrochelatase which catalyses the last step of heme synthesis where normally it incorporates Fe to protoporphyrin IX to produce heme. In Pb toxicity Zn will be incorporated in place of Fe to protoporphyrin resulting in the production and accumulation of ZPP [36]. However, in the abundance of Hb, even in serious case of Pb intoxication, increased ZPP is relatively harmless because it may constitute less than 1 % of the total Hb production [37].

Pb is known to generate free radicals at different levels. Inhibition of delta ALAD by Pb results in accumulation of delta ALA that can be rapidly oxidized to free radicals such as superoxide anion, hydroxyl radical and hydrogen peroxide [38]. Pb is also known to have the capacity to stimulate ferrous ion initiated membrane lipid peroxidation [39]. Several antioxidant molecules such as GSH and GSSG levels and the activities of antioxidant enzyme levels such as SOD, CAT, glutathione peroxidase (GPx) and glutathione reductase (GR) are most commonly used parameters to evaluate Pb induced oxidative damage [38,

Fig. 1 Tree diagram showing similarity among variables of all pregnant women ($n = 175$)

Fig. 2 Tree diagram showing similarity among groups of all pregnant women ($n = 175$)

40]. GSH plays a pivotal role in protection of cells against oxidative stress. It can act as a non-enzymatic antioxidant by direct interaction of SH group with ROS or it can be involved in the enzymatic detoxification reactions for ROS as a cofactor or a coenzyme [41]. Many studies have shown decrease in GSH levels during Pb toxicity [39], similarly in our study we also observed significant depletion of GSH in anemic women compared to controls. Significant change was also observed in the oxidized form of GSH, i.e., GSSG. CAT and SOD are metalloproteins and accomplish their antioxidant functions by enzymatically detoxifying the peroxides ($-OOH$), H_2O_2 and O_2^- respectively. CAT has been suggested to provide important pathway for H_2O_2 decomposition into H_2O and O_2 at high steady state H_2O_2 concentration, whereas SOD removes the superoxide ions into H_2O_2 and needs copper and Zn for its activity. There are conflicting reports regarding influence of Pb on SOD and CAT activities. Some studies showed decreased activities of SOD and CAT [42], whereas other studies showed their increased activities [43]. In our study we

observed a significant decrease in CAT and SOD activities in Fe deficient pregnant anemic women when compared with controls (Table 3). The decreased activities of CAT and SOD may be explained in part mainly due to interaction of Pb with essential metals such as copper, Zn, Fe and induction of free radical. As stated earlier copper and Zn are essential cofactors for SOD. CAT contains heme as the prosthetic group; the biosynthesis of which is inhibited by Pb [44]. Additionally, we also found that TAC was low in Fe deficient pregnant anemic women. TAC considers the cumulative action of all the antioxidants present in the RBC and body fluids and provides an integrated parameter rather than the simple sum of measurable antioxidants.

Pb inheritance facilitates conversion of Hb into metHb. This reaction is possible not only in pure Hb solution but also in lysate, where in antioxidant defense systems are present. It seems that during Hb oxidation in the presence of Pb, H_2O_2 is generated, which may induce lipid peroxidation in erythrocyte cell membranes [45]. A significantly increased lipid peroxidation was also observed in our study.

Concluding it may be stated that even relatively low BLLs are associated with elevated risk of mild, moderate and even severe Fe deficiency anemia. Moreover, perturbation of prooxidants and antioxidants in pregnant anemic women indicate definite oxidative stress, which may be due to Pb intoxication. Since Pb pollution and bioavailability of Pb by other means can be controlled and steps can be taken to reduce the prevalence of anemia during pregnancy, regulatory and health agencies should consider this as a priority and make more substantial efforts towards resolving this public health problem.

References

1. Caroline Ros B, Lillian M. Lead exposure, interactions and toxicity: food for thought. *Asia Pacific J Clin Nutr.* 2003;12:388–95.
2. Courtois E, Marques M, Barrientos A. Lead-induced down-regulation of soluble guanylate cyclase in isolated rat aortic segments mediated by reactive oxygen species and cyclo-oxygenase-2. *J Am Soc Nephrol.* 2003;14:1464–70.
3. Ahamed M, Verma S, Kumar A, Siddiqui MKJ. Environmental exposure to lead and its correlation with biochemical indices in children. *Sci Total Environ.* 2005;346:48–55.
4. Donaldson WE, Knowles SO. Is lead toxicosis a reflection of altered fatty acid composition of membrane? *Comp Biochem Physiol C.* 1993;104:377–9.
5. Leggett RW. An age specific kinetic model of lead metabolism in humans. *Environ Health Perspect.* 1993;101:598–616.
6. Menke A, Muntner P, Batuman P, Silbergeld EK, Guallar E. Blood lead below 0.48 µg/dl (10 µg/dl) and mortality among US adults. *Circulation.* 2006;114:1388–94.
7. Ong CN, Phoon WO, Law HY, Tye CY, Lim HH. Concentrations of lead in maternal blood, cord blood and breast milk. *Arch Dis Child.* 1985;60:756–9.
8. Roels H, Hubermont G, Buchet JP, Lauwerys R. Placental transfer of lead, mercury, cadmium, and carbon monoxide in women. III. Factors influencing the accumulation of heavy metals in the placenta and the relationship between metal concentration in the placenta and in maternal and cord blood. *Environ Res.* 1978;16:236–47.
9. Foster WG. Reproductive toxicity of chronic lead exposure in the female cynomolgus monkey. *Reprod Toxicol.* 1992;6:123–31.
10. Sierra EM, Tiffany-Castiglioni E. Effects of low-level lead exposure on hypothalamic hormones and serum progesterone levels in pregnant guinea pigs. *Toxicology.* 1992;72:89–97.
11. Moser R, Oberley TD, Daggett DA, Friedman AL, Johnson JA, Siegel FL. Effects of lead administration on developing rat kidney. I. Glutathione S-transferase isoenzymes. *Toxicol Appl Pharmacol.* 1995;131:85–93.
12. Corpas I, Gaspar I, Martínez S, Codesal J, Candelas S, Antonio MT. Testicular alterations in rats due to gestational and early lactational administration of lead. *Reprod Toxicol.* 1995;9:307–13.
13. Buchheim K, Noack S, Stoltenburg G, Lilienthal H, Winneke G. Developmental delay of astrocytes in hippocampus of rhesus monkeys reflects the effect of pre and postnatal chronic low level lead exposure. *Neurotoxicology.* 1994;15:665–9.
14. Andrews KW, Savitz DA, Hertz-Picciotto I. Prenatal lead exposure in relation to gestational age and birth weight: a review of epidemiologic studies. *Am J Ind Med.* 1994;26:13–32.
15. Bellinger D, Leviton A, Allred E, Rabinowitz M. Pre- and postnatal lead exposure and behavior problems in school-aged children. *Environ Res.* 1994;66:12–30.
16. Rothenberg SJ, Poblano A, Garza-Morales S. Prenatal and perinatal low level lead exposure alters brainstem auditory evoked responses in infants. *Neurotoxicology.* 1994;15:695–9.
17. West WL, Knight EM, Edwards CH, Manning M, Spurlock B, James H, et al. Maternal low level lead and pregnancy outcomes. *J Nutr.* 1994;124(98):1S–6S.
18. Bogden JD, Kemp FW, Han S, Murphy M, Fraiman M, Czerniach D. Dietary calcium and lead interact to modify maternal blood pressure, erythropoiesis, and fetal and neonatal growth in rats during pregnancy and lactation. *J Nutr.* 1995;125:990–1002.
19. Kristensen P, Eilertsen E, Einarsdottir E, Haugen A, Skaug V, Ovrebo S. Fertility in mice after prenatal exposure to benzo[a]pyrene and inorganic lead. *Environ Health Perspect.* 1995;103:588–90.
20. Mahaffey KR. Environmental lead toxicity: nutritional as a component of intervention. *Environ Health Perspect.* 1990;89:75–8.
21. Kwong WT, Friello P, Semba RD. Interactions between iron deficiency and lead poisoning: epidemiology and pathogenesis. *Sci Total Environ.* 2004;330:21–37.
22. Conrad ME. Newly identified iron-binding protein in human duodenal mucosa. *Blood.* 1992;79:244–7.
23. World Health Organization (WHO)/United Nations Children's Fund/United Nations University. Iron deficiency: indicators for assessment and strategies for prevention. Geneva: WHO, 1998.
24. International Nutritional Anemia Consultative Group. Measurements of iron status. Washington: INACG; 1985.
25. Kaneko JJ, editor. Clinical biochemistry of domestic animals. 4th ed. New York: Academic; 1999.
26. Berlin A, Schaller KH. European standardized method for the determination of aminolevulinic acid dehydratase activity a blood. *Zeitsch Klin Chem Klin Biochim.* 1974;12:389–90.
27. Blumberg WE, Eisinger J, Lamola AA, Zuckerman DM. The hematofluorometer. *Clin Chem.* 1977;23:270–4.
28. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem.* 1959;82:70–7.
29. Ohkawa H, Oshiba N, Yagi K. Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anat Biochem.* 1979;95:351–8.
30. Aebi H. Catalase in vitro. *Methods Enzymol.* 1984;105:121–6.
31. Mc Cord JM, Fridovich I. Superoxide dismutase: an enzyme functions for erythrocyte. *J Biol Chem.* 1969;244:6049–55.
32. Lowry OH, Rosenbrough NJ, Farr AL, Randell RJ. Protein measurement with folin–phenol reagent. *J Biol Chem.* 1951;193:265–75.
33. Bernin Iris FF, Stain JJ. Ferric reducing ability of plasma [FRAP] as a measure of antioxidant power, The FRAP Assay. *Anal Biochem.* 1996;239:70–6.
34. Austrin KH, Bishop DF, Wetmur JG, Kaul BC, Davidow B, Desnick RJ. Aminolevulinic acid dehydratase isozymes and lead toxicity. *Ann NY Acad Sci.* 1987;514:23–9.
35. 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.
36. Marcus AH, Schwartz J. Dose-response curves for erythrocyte porphyrin vs. blood lead: effects of iron status. *Environ Res.* 1987;44:221–7.
37. Onalaja VO, Claudio L. Genetic susceptibility to lead poisoning. *Environ Health Perspect.* 2000;108:23–8.
38. Gurer-Orhan H, Sabir HU, Ozgunes H. Correlation between clinical indicator of lead poisoning and oxidative stress parameters in controls and lead exposed workers. *Toxicology.* 2004;195:147–54.

39. Bechara EJH. Lead poisoning and oxidative stress. Institute of de Quimica, Universidade de Sao Paulo, Brazil. SFRR's 12th biennial meeting programme and abstracts, 5–9 May 2004, Crown Plaza, Panamericano Hotel, Buenos Aires, Argentina, S9–46, vol. 36. Free Radic Biol Med; 2004.
40. Kasperczyk S, Birkner E, Kasperczyk A, Kasperczyk J. Lipids, lipid peroxidation and 7-ketocholesterol in workers exposed to lead. *Hum Exp Toxicol*. 2005;24:287–95.
41. Ding Y, Gonick HC, Vaziri ND. Lead promotes hydroxyl radical generation and lipid peroxidation in cultured aortic endothelial cells. *Am J Hypertens*. 2000;13:552–5.
42. Valenzuela A, Lefauconnier JM, Chaudiere J, Bourre JM. Effects of lead acetate on cerebral glutathione peroxidase and catalase in the suckling rat. *Neurotoxicology*. 1989;10:63–9.
43. Ahamed M, Verma S, Kumar A, Siddiqui MKJ. Delta-amino-levulinic acid dehydratase inhibition and oxidative stress in relation to blood lead among urban adolescents. *Hum Exp Toxicol*. 2006;25:547–53.
44. Patil AJ, Bhagwat VR, Patil JA, Dongre NN, Ambekar JG, Jailkhani R, Das KK. Effect of lead (Pb) exposure on the activity of superoxide dismutase and catalase in battery manufacturing workers (BMW) of Western Maharashtra (India) with Reference to Heme Biosynthesis. *Int J Environ Res Public Health*. 2006; 3:329–37.
45. Vargas H, Castillo C, Posadas F, Escalante B. Acute lead exposure induces renal heme oxygenase-1 and decreases urinary Na⁺ excretion. *Hum Exp Toxicol*. 2003;22:237–44.