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Effect of Lead on Human Blood Antioxidant Enzymes and Glutathione

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PJJ and PS designed the study, wrote the protocol and supervised the work. Authors APJ and DY carried out all laboratories work. Author VPSS performed the statistical analysis. Author HP managed the analyses of the study. Author APJ managed the literature searches and wrote the first draft of the manuscript. Author PJJ edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Human exposure to lead may alter the enzyme antioxidants level and the interaction between antioxidants and blood lead level (BLL) eventually cause oxidative stress. Therefore, the present investigation was carried out to establish the relationship between the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and antioxidant

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molecule reduced glutathione (GSH) with varying BLLs. A randomly selected 250 subjects from rural-urban populations of either sex ranging in age from 20 to 70 years were investigated. The mean value of BLL in 250 subjects was 15.16±11.82 µg/dl with a minimum level of 0.1 µg/dl and the maximum level of 39.71 µg/dl. Pearson's linear correlation analyses were used to evaluate the influence of BLL on the enzyme antioxidants. Significant modulation of enzymes antioxidants on BLLs and characteristics of demographic data such as habits, substances abuse (smoking effect), sex and diet of rural-urban population were observed in present investigation. The urban population, non-vegetarians, males and smokers had higher blood lead levels. The BLL was negatively correlated with the activity of SOD, CAT, GPx and GSH. Increased BLLs and statistically significant decreased activity of enzyme antioxidants might contribute to lead-induced toxicity in urban population. These findings suggest a rationale for lowering the enzyme antioxidants with decreasing BLLs.

Keywords: Blood lead level; superoxide dismutase; glutathione peroxidase; catalase; reduced glutathione.

1. INTRODUCTION

Environmental and occupational lead pollution substantially increases in both developed and developing countries. In many developing countries lead contamination is increasing rapidly and their effects on human health are expected to increase [1]. Scientific evidence show that lead retards the mental and physical development of children, causing reading and learning disabilities, changes in behavior, such as hyper activity, reduced attention span and learning loss, even at low level of exposure [2]. Ahamed et al. [3] evaluated the effects of blood lead levels (BLLs) on oxidative stress parameters in children suffering from neurological disorders. Lead also induces changes in the composition of erythrocyte membrane proteins and lipids [4], and inhibits the hemoglobin synthesis [5]. In some human studies disrupted prooxidant/antioxidant system was demonstrated in lead exposed workers [6,7]. Lead associated hypertension was also attributed to increased generation of reactive oxygen species [8]. Lead poisoning has also been reported to disrupt many biological structures and functions, including those of the auditory system [9]. Sugawara et al. [7] reported that the lowered concentration of GSH and decreased activity of SOD, CAT and GPx in erythrocytes from workers exposed to lead may play a part in the increased membrane lipid peroxidation. Chiba et al. [10] reported that the indices of lead exposure in blood and urine of lead exposed workers found significant correlation between the activities of SOD, GSH, CAT and the lead. Depressed levels of glutathione reductase, glutathione peroxidase, and glutathione-S-transferase were found to correlate with depressed glutathione levels in occupationally lead exposed workers [11]. Jangid et al. [12] reported that BLL determinations remain the most suitable method for monitoring recent lead toxicity. A systematic assessment of BLL in children and associated risk factors in China was conducted by Wang, et al. [13] by reviewing 388 relevant original articles. They found that the overall geometric mean BLL was 71 µg/L and the prevalence of elevated BLL was 18.48% among children. Etchevers, et al. [14,15] conducted a national study in 2008-2009, to determine the BLL distribution in children between the ages of six months and six years in France. According to the study the geometric mean BLL was 14.9 µg/l and 0.09% of the children had BLLs exceeding 100 µg/l, 1.5% exceeding 50 µg/l.

Enzymatic antioxidants like SOD, CAT, and GPX are produced endogenously in the cells, whereas non enzymatic antioxidants like carotenoids, flavonoids, vitamins, minerals, etc. are constituents of many fruits, vegetables, nuts, grains and some meats [16]. The amount of antioxidants present under normal physiological conditions is just adequate to quench the free radicals that are generated at a normal physiological rate. Any further increment in the concentration of free radicals due to environmental or natural causes can create an imbalance between the free radicals and antioxidants leading to oxidative stress [17].

The present study investigated the importance of enzyme antioxidants in lead-induced toxicity in hematological systems of human being. To achieve this goal selected enzyme antioxidants level were determined along with BLLs of a rural-urban subjects of Jaipur.

2. MATERIALS AND METHODS

2.1 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 urban and rural population of Jaipur. A written consent was obtained from all 250 subjects. The information from subjects were obtained with the help of a questionnaire filled by face-to-face interviews. Questionnaire included 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.

2.2 Collection of Blood Sample

Blood sample from each subject was collected in forenoon from the ante cubital vein using aseptic techniques at Central Laboratory, Department of Biochemistry, S.M.S. Medical College, Jaipur. Each samples consisted of 5 ml. of blood which was collected in a di-potassium ethylene diamine tetra acetic acid (EDTA) vial and investigations were performed on this sample.

2.3 Biochemical Assay

2.3.1 Blood lead (Pb)

Blood sample collected from the subjects were analyzed for lead level by Atomic Absorption Spectrophotometer (AAS) at 283.3 nm according to the method of Fernandez, [18]. Sample pre-treatment consisted of a dilution with a dilute surfactant. The method is directly calibrated with lead standards prepared in dilute HNO_3.

2.3.2 Superoxide dismutase (SOD)

SOD activity was measured spectrophotometrically at 505 nm at 37°C according to the method of Marklund and Marklund, [19]. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-indophenyl)-3-(-4nitrophenol)-5-phenyltetrazoliumchloride (INT) to form a red formazen dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay. The activity was expressed as Units/g Hemoglobin.

2.3.3 Glutathione peroxidase (GPX)

GPX activity was measured spectrophotometrically at wavelength of 340 nm by the method given by Pagalia and Valentine, [20]. Glutathione Peroxidase (GPX) catalyses the oxidation of Glutathione (GSH) by Cumene Hydroperoxide into GSSG. In the presence of Glutathione Reductase (GR) and NADPH the oxidized Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+.

2.3.4 Catalase (CAT)

CAT activity was measured in hemolysates at 30°C according to the method given by Aebi, [21]. The rate of decomposition of H_2O_2 by catalase is measured spectrophotometrically at 240 nm because H_2O_2 absorbs light at this wavelength. In the ultraviolet range H_2O_2 shows continual increase in absorption with decreasing wavelength. The decomposition of H_2O_2 can be followed directly by the extinction at 240 nm. The difference in extinction per unit time is the measure of the catalase activity.

2.3.5 Reduced glutathione (GSH)

GSH activity was measured according to the method of Beutler [22]. DTNB {5, 5'-Dithiobis (2-nitrobenzoic acid)} is a disulfide compound which is readily reduced by glutathione, forming a highly colored yellow anion. The optical density of this yellow substance is measured at 412 nm by spectrophotometer.

2.4 Statistical Analysis

Statistical analysis was done using statistical software SPSS 10. One way analysis of variance (ANOVA) and Tukey's test was applied to establish the significance of the observed differences among various groups. F-test was also carried out to establish the significant differences in the demographic character and habits group. Pearson's correlation was carried out wherever applicable.

3. RESULTS

All the 250 subjects selected in the present study were divided in to four groups as group-A, group-B, group-C and group-D depending upon the blood lead concentration. The subjects belonging to Group-A (n=103, 41.00%) had BLLs between 0-10 µg/dL and this group was considered to be the safest as per CDC and WHO guidelines (CDC, 1991 and WHO, 1995). In group-B (n=67, 27.00%), the Lead level was 10.01-20 µg/dL whereas in group-C (n=53, 21.00%) and group-D (n=27, 11.00%) it was 20.01-30 µg/dL and 30.01-40 µg/dL respectively (Table 1). Table 2 shows the BLL and biochemical characteristics (mean±SD) of selected subjects as per demographic groups such as sex, rural-urban, diet and substances abuse (smoking). Relatively low BLL and a significantly high activity of SOD, CAT and GSH were reordered in females as compared to males. No significant difference for GPx activity was recorded in females from males. A significantly higher BLL was recorded in urban subjects as compared to rural subjects. In urban subjects, a significantly low activity of SOD and GSH was recorded as compared to rural subjects. A significantly low BLL and lower SOD, GPx and GSH activity were recorded in non-vegetarian subjects as compared to vegetarians whereas no significant difference for CAT activity was recorded. BLL was low in non-smokers as compared to smokers. Nonsmokers had significantly high activity of SOD, CAT and GSH as compared to smokers. The variable influence of BLLs on SOD, CAT, GPx and GSH of various subjects groups has been presented in Table 3. SOD activity was significantly different (P<0.001) in various groups and the maximum activity was recorded in group-A, whereas the least SOD activity was recorded in group-D having maximum BLLs. Significantly lower activity of GPx was recorded as compared to other groups. CAT activity was also affected significantly (P<0.001) by BLLs. Similar observations were made for GSH also. All the four enzymes investigated here exhibited a significantly low activity in group-D subjects having maximum BLLs. The relationship between BLLs and various antioxidant enzymes studied has been presented in Table 4 and Fig. 1. A significant negative correlation (Fig. 1) of BLL and the activity of SOD (r= - 0.699), GPx (r= - 0.065), CAT (r= - 0.199) and GSH (r= - 0.136) were observed in the present study.

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	$3.60^{\pm}2.71$
Group-B (67)	10.01-20 µg/dL	15.21±2.65
Group-C (53)	20.01-30 µg/dL	26.82±2.53
Group-D (27)	30.01-40 µg/dL	36.38±2.83

Table 2. Selected enzyme antioxidants in subjects with varying demographic habits

Subjects	Enzyme antioxidants				
	Lead (µg/dl)	SOD (U/g Hb)	GPx (U/g Hb)	CAT (K/g Hb)	GSH (mg/dl)
Male (n=217)	15.55*±11.88	801.90±262.66	41.01±12.51	270.07±57.17	39.01±8.32
Female (n=33)	11.56±10.86	916.08*± 249.60	40.18^{NS}± 12.03	306.37*±77.22	42.28*±11.13
Rural (n=141)	8.93±7.18	912.23±249.23	40.05±12.32	278.74±55.07	41.05±13.12
Urban (n=109)	23.23*±9.80	692.92*± 228.10	42.01^{NS}±12.54	269.84^{NS}± 68.38	37.01*±7.54
Vegetarian (n=153)	12.65±10.34	836.13±261.15	41.52±11.87	274.94±56.48	40.42±10.65
Non-Veg. (n=97)	19.13*±12.91	785.83*± 265.27	39.93*± 13.25	274.73^{NS}±68.44	37.23*±8.25
Smokers (n=139)	17.16*±12.92	779.85±264.10	40.94±12.22	271.12±61.15	38.74±7.28
Non-Smokers (n=111)	11.56±10.86	916.08*± 249.60	40.18^{NS}±12.03	306.37*±77.22	40.88*±11.53

*NS = Not significantly different, *= significantly different according to F-test at P<0.05*

Table 3. Variable influence of selected enzyme antioxidants in various subjects groups

Parameters	Subject group (Mean ± SD)			
	A(0-10 µg/dl) (n=103)	B(10.01-20 µg/dl) (n=67)	C(20.01-30 µg/dl) (n=53)	D(30.01-40 µg/dl) (n=27)
SOD (U/g Hb)	1010.09[a]±205.75 (969.88-1050.30)	767.23[b]±176.65 (724.14-810.31)	634.03[c] ±211.99 (575.60-692.46)	559.45[d] ±194.68 (482.43-636.46)
GPx (U/g Hb)	41.06 [a] ±11.13 (38.88-43.23)	39.83 [a] ±14.32 (36.34-43.32)	45.84 [b] ±10.62 (42.92-48.77)	33.25[d]±11.52 (28.69-37.81)
CAT (K/g Hb)	293.32 [a] ±56.13 (282.32-304.29)	245.33[b]±29.77 (238.07-252.59)	293.27[a]±68.69 (274.33-312.20)	241.61[d]±80.36 (209.82-273.40)
GSH (mg/dl)	40.93[a] ±11.13 (38.88-41.43)	39.83[a] ±14.32 (36.34-43.32)	36.97[b]±5.69 (33.82-38.77)	33.25[d] ±11.52 (28.69-37.81)

Values represent Mean±SD (n=250) and 95% CI for mean. Mean values with different letter in superscript in a row are significantly different according to Tukey's multiple comparison procedure

4. DISCUSSION

Lead toxicity is one of the major environmental hazards in the world. Lead induces oxidative stress, and may deteriorate biological macromolecules either by increased production of reactive oxygen species such as superoxide and hydroxyl radicals or by depletion of the cell's major antioxidants [23]. Therefore the present study demonstrated the BLL and its impact on various antioxidant enzymes such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GP_X) and endogenous antioxidant reduced glutathione (GSH).

The significantly higher BLL in the males and smokers as compared to females and non-smokers can be attributed to the fact that in the developing countries like India, males and the smokers are relatively more exposed to pollution via variety of occupations as compared to females and non-smokers. No precise investigation on effect of gender on oxidative stress with reference to lead has been put forward till date. Elevated lead concentration in blood in urban population can be ascribed to increased exposure to various sources of lead which includes pollution through paints [24], lead recycling, presence of lead in cosmetics [25,26] and in public drinking water supply in lead pipes [27,28]. The BLL was significantly higher in non-vegetarians as compared to vegetarians. The reason for this variability remains unclear and requires a more systematic study regarding the quality of non-vegetarian food in terms of heavy metals or lead content and also the frequency of consumption of non-vegetarian food.

SOD is the most important enzyme involved in the antioxidant defense and responsible for scavenging superoxide free radical (O^{2-}) catalyzing a dismutation reaction and converting it into H_2O_2. Superoxide radical is formed during various chemical reactions by the addition of an extra electron to the oxygen molecule and making it highly reactive. It reacts with various molecules and results in either direct damage or generation of potentially harmful products like H_2O_2. The load of H_2O_2 synthesized by SOD activity is removed constantly and efficiently by another enzymatic antioxidant glutathione peroxidase present in the same sub-cellular compartment as SOD, therefore their activities are interlinked [29]. The present investigation showed the decrement in SOD activity was paralleled with increment in BLLs in subjects ($P<0.001$, Fig. 1, Table 4). A negative correlation between SOD and BLL was recorded (Fig. 1). Reports on the influence of lead on SOD activity are divergent in animal studies [30,31]. A decreased SOD activity in erythrocytes under the influence of lead has been reported earlier also [32, 33, 34, 3]. Glutathione peroxidase, selenium containing metallo enzymes, terminates the chain reaction of lipids peroxidation by removing lipid hydro peroxide from the cell membrane. GPx is most important in removing H_2O_2 because it is in the same sub cellular compartment (cytosol and mitochondria) as in the case SOD. It is the only human enzyme requiring the element selenium for its activity and a selenocysteine residue (-SeH) is present at its active site [29]. It was observed that the decrement in GPx concentration was inversely proportional with the increment in BLLs in subjects ($P<0.001$, Table 4). There was a negative correlation between GPx and BLLs which is evident from the coefficient of correlation value of $r= -0.065$ (Fig. 1, Table 4).

Decrement in GPx level in response to lead concentration is possibly due to free radical

damage by lead and resulted in ROS generation. Therefore, prooxidant/antioxidants balance is disrupted. Kasperczyk et al. [35] reported that the activity of GPx significantly increased in low blood lead group when compared to control group and decreased when compared to high blood lead level group.

Sugawara et al. [7] reported that among the antioxidant enzymes GPx is the most sensitive enzyme. They studied the incubation of erythrocytes in the presence of 100 µg/dl lead and found that the inhibition of activity of SOD was 14%, catalase was 10% and GPx was 35%. Hunaiti and Soud, [36] also reported a concentration-dependent decrease in the activity of GPx. This reduction was up to 50%, in the presence of 100–400 µg/dl lead. The activity of GPx has also been studied in blood from people exposed to Lead. Monteiro et al. [6] found that among three populations exposed to lead in Brazil, in two cases there were increases of activity of GPx in erythrocytes, and in one case, there was a decrease of activity of this enzyme. Similarly, Solliway et al. [37] obtained a significant dose-dependent elevations in the activity of erythrocyte GPx (31%) in people exposed to Lead (mean BLL=40.7µg/dl). However, Wasowicz et al. [38] analyzed a population with mean BLL=50.4 µg/dl and observed the decreasing activity of GPx in serum (9%) as well as in erythrocytes (18%).

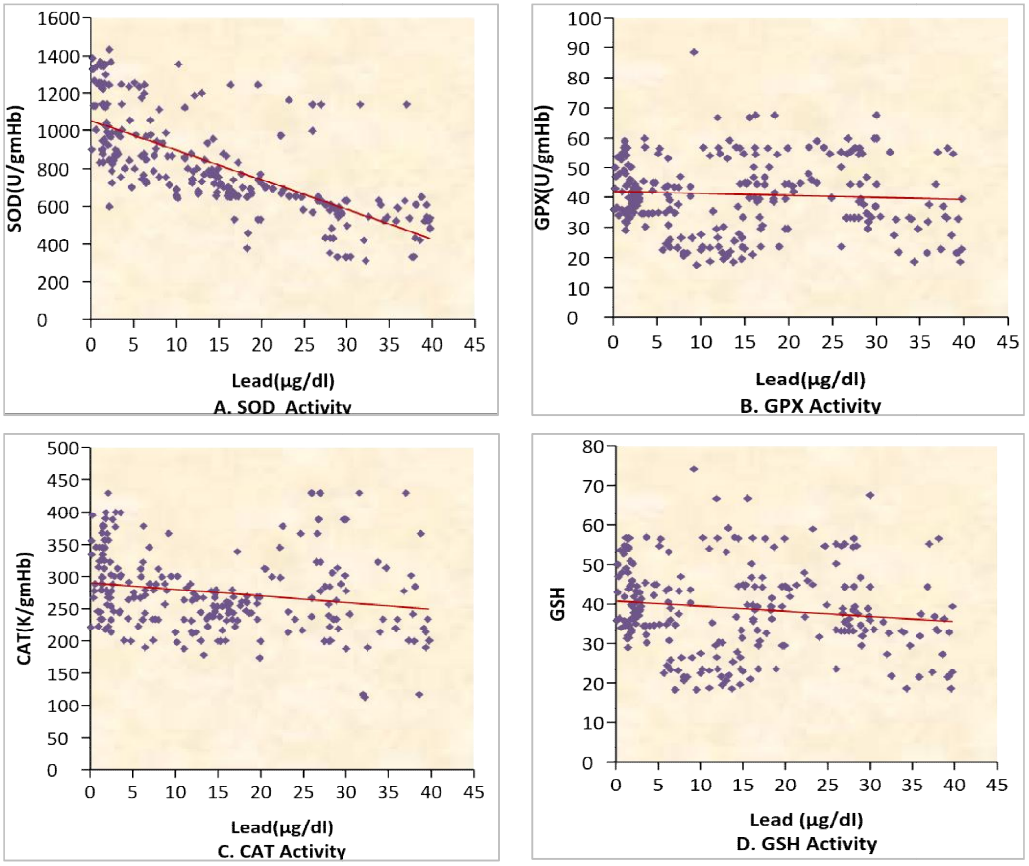

Fig.1. (A-D) Correlation between various antioxidant enzymes and blood lead level (BLL)

Table 4. Correlation between selected enzymes antioxidants and Lead in various subjects

Correlation	SOD (U/g Hb)	GPx (U/g Hb)	CAT (K/g Hb)	GSH (mg/dl)
Lead (Pb) (µg/dl)	r= - 0.699, P<0.001	r = -0.065, P<0.01	r= - 0.199, P<0.01	r= - 0.136, P<0.001

n =250. r= correlation coefficient (Pearson's correlation)

Reduction in CAT concentration was recorded with increasing BLLs (P<0.001, Table 4, Fig. 1, Table 4). Research on influence of Lead on CAT activity has given divergent results [39,40]. In the present study CAT activity was low in subjects associated with increased BLLs. This may be due to the inhibitory action of lead (Pb) on CAT [41]. The CAT and the SOD are metalloproteins and accomplish their antioxidant functions by enzymatically detoxifying the peroxides (OH, H_2O_2) and superoxide anion. Previous investigations on this aspect have not put forth any conclusive answer as few of the studies revealed decreased activities of SOD and CAT [42,43], and on the contrary, few others have showed increased activities of these enzymes [44,45]. In the present investigation a significant decrease in CAT and SOD activities was observed. Correlation analysis showed a negative correlation between BLL and CAT, suggesting that increased blood lead levels and decreased activities of antioxidant enzymes are related. The decreased activities of the CAT and SOD may be due to the interaction between lead and essential metals such as Copper, Zinc, and Iron. Copper and Zinc are essential cofactors for SOD, whereas CAT also contains haem as the prosthetic group, the biosynthesis of which is inhibited by lead [46,47]. Antioxidant molecule such as glutathione (GSH) and antioxidant enzymes such as SOD, CAT and glutathione peroxidase (GPx) are commonly studied to evaluate lead-induced oxidative damage [48,49]. Reduced glutathione (GSH) is an endogenous antioxidant. Increment in BLLs was inversely proportional with decline in GSH concentration (P<0.001, Table 4). There was a negative correlation (r= -0.136) between GSH and BLLs was recorded (Table 2, Fig. 1). Influence of lead on GSH activity has not been clearly established till date. A reduction in GSH levels during lead toxicity has been reported in many studies [7,50]. Findings of the present study are also in accordance to the previous reports.

5. CONCLUSION

Lead is an important environmental toxicant in developed and developing nations. It may cause serious health problems. The urban population, non-vegetarians, males and smokers had more blood lead levels. A significant reduction in these antioxidant enzyme activities were recorded in higher BLLs subjects. Reactive oxygen species are involved in various cellular processes like growth, progression, differentiation and death. Low concentration of reactive oxygen species may be beneficial in intracellular signaling and defense against microorganism. Nevertheless, higher concentrations of reactive oxygen species play role in the aging process as well as in a number of human disease states, including cancer, ischemia, and failures in immunity and endocrine functions. As a safeguard against the accumulation of reactive oxygen species, several non-enzymatic and enzymatic antioxidant activities exist. Therefore, when oxidative stress arises as a consequence of a pathologic event, a defense system promotes the regulation and expression of these enzymes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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