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Individual susceptibility and genotoxicity in workers exposed to hazardous materials like lead

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ABSTRACT

The present study was undertaken to investigate lead-induced toxicity in occupationally exposed humans and to evaluate whether genetic damage can be correlated with the known clinical indicators of lead poisoning. For this purpose, genotoxicity biomarkers along with some clinical indices of lead poisoning were determined in blood samples of battery plant workers and compared with healthy control subjects. Workers had significantly increased chromosomal aberrations, micronuclei and DNA damage compared to the controls. Increased blood lead levels (BLLs), decreased hemoglobin, PCV and symptoms of lead poisoning were used as clinical indices of lead toxicity. In addition gene polymorphisms in ALAD and MGP gene were investigated and correlated with BLL and hemoglobin content. Our results showed no significant effects of the ALAD G177C polymorphism on BLL concentrations and BLL concentrations varied to levels much above the normal reference ranges independent of the genotype. Although, significance could not be achieved, ALAD 1-2/2-2 type subjects had numerically higher BLLs (76.2–89.1 μg/dl), compared to ALAD 1-1 volunteers (21.8–79.1 μg/dl).Similarly, this study also aimed to identify the relation of some SNPs with emphasis on lead toxicity and since MGP gene is an important biomarker associated with calcium metabolism; it was hypothesized that it may be associated with lead toxicity. However, we did not find any significant association of MGP T-138C and lead poisoning. Further studies on the role of gene polymorphisms over a larger population along with genotoxicity parameters and biochemical analyses may serve to understand lead toxicity.

1. Introduction

Although, currently available scientific literature reports the molecular and biochemical effects of lead in adults, children and infants, understanding the mechanisms of lead toxicity still remains a major challenge to researchers [1]. Lead exposure has been associated with increased risk of lung, stomach, and bladder cancer in diverse human populations [2–4]. In developing countries, industrial workers are easily prone to the toxic effects of lead due to lack of the knowledge about its safe handling [5].

Reported genetic effects of lead include the ability of this metal to cause geno and cytotoxicity. Several in vitro and in vivo studies have investigated the genotoxic effects of lead. While some studies showed an increased chromosomal aberrations (CA) and/or sister chromatid exchange (SCE) frequency in lymphocytes from workers exposed to lead [6,7]; others reported negative results [8].

Biochemical effects of lead have been previously investigated and it has been proven that since lead is a divalent ion, it can also inhibit the functioning of important proteins which use divalent ions like calcium and zinc as co-factors [9–12]. Lead is a potential inhibitor of heme, and is a direct contributor to anemia in exposed individuals. The metal also binds strongly to proteins involved in heme biosynthesis like aminolevulinic acid dehydratase (ALAD) and heme synthetase [8].

In addition, individual differences in response to xenobiotics are often caused by genetic differences that result in altered rates of biotransformation (metabolism). Individuals differ in their ability to detoxify and eliminate xenobiotics [7]. Gene–environment interactions that link exposures, polymorphisms, and disease states are useful in interpreting susceptibility to lead toxicity. Lead is a potent inhibitor of δ-aminolevulinic acid dehydratase (ALAD), and has been widely acknowledged to play an important role in the pathogenesis of lead poisoning [13]. A polymorphism at position 177 leading to a G → C transversion results in occurrence of two alleles (ALAD-1 and ALAD-2) and three isozyme phenotypes, ALAD 1-1, ALAD 1-2, and ALAD 2-2. Studies have suggested that carriers of the ALAD-2 allele have higher blood lead levels (BLL) than ALAD-1 subjects and thus are more susceptible to lead toxicity [14–16].

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Some authors suggest that the enhanced capacity of ALAD-2 to bind lead may confer resistance to its harmful effects because subjects with ALAD-2 may have less bio-available lead [15,17].

However, controversial results with respect to the role of this gene and lead poisoning have been observed across different populations. Also, genetic differences can also be attributed to SNPs present in other genes which may indirectly or directly influence the functioning of proteins. This study therefore, tries to explore the relationship between polymorphisms in ALAD and MGP genes and investigate their probable association with lead poisoning. Human MGP (Matrix γ-carboxyglutamic acid protein) gene is located at 12p13.1–p12.3 which codes for a 10-kDa Matrix Gla (γ-carboxyglutamic acid) protein [18–20]. Currently available scientific evidence indicates that MGP plays a significant role as an inhibitor of mineralization [18,19]. MGP gene suppresses calcium ion function in the cartilage, and other soft tissues, in addition, lead and calcium are divalent cations, having the same absorption pathways [10]. Therefore, Pb^{2+} ions can compete with Ca^{2+}; the influence of MGP polymorphism with respect to lead deposition assumes importance in understanding the molecular basis of lead toxicity.

The present study was taken up to understand the genetic, molecular and biochemical effects of lead on the human system. The methodology employs genotoxicity estimations (CA), Micronucleus Test (MNT), DNA damage (SCGE), polymorphism analyses (ALAD, MGP); hemoglobin, CBP and blood lead estimations to have an overall understanding of the potential risk of health problems in battery manufacturing workers due to lead poisoning when compared to unexposed subjects (controls).

2. Materials and methods

2.1. Collection of samples

215 volunteers (total 198 men and 27 women) aged from 18 to 51 years living in the city of Hyderabad, Andhra Pradesh, India were enrolled. 113 subjects were working occupationally in lead battery industry. Only subjects reporting at least two of the symptoms of lead toxicity like headache, nausea, gastritis, vomiting, lethargy and poor appetite were enrolled in the study. 102 volunteers not exposed occupationally to lead formed the controls. This study was approved by the Ethics Committee, and each subject provided written informed consent. Details of previous medical history, present health status, nutritional status, years of exposure and duration of working hours were recorded. 5 ml of venous blood samples were collected from each volunteer in two tubes one with heparin for metal analysis, and one containing EDTA for hematological evaluations. Before the collection, the skin of the volunteer was cleaned with alcohol and ultrapure laboratory grade Milli-Q-water (Millipore Systems).

2.2. Biochemical assessments

2.2.1. Determination of blood lead levels and hematological parameters

For the estimation of BLLs, ESA Model 3010B Lead analyzer was used, which determines the level of lead in blood by anode stripping voltammetry (ASV) [21]. Experiments were performed at Secunderabad Diagnostic Centre, Hyderabad, A.P., which is accredited by the National Accreditation Board for Testing and Calibration Laboratories (NABL) for following ISO/IEC 17025 Standards. Complete blood picture was determined using ADVIA Cell counter for each sample. This included hemoglobin, platelet count, total white blood cell (WBC) count, total red blood cell (RBC) count, packed cell volume (PCV) and mean corpuscular volume (MCV).

2.3. Molecular testing

2.3.1. ALAD and MGP gene polymorphism

An assay based on polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) was used to determine the genotype of ALAD [15,17] and MGP gene [19]. PCR was performed in a 50 μl reaction volume using genomic DNA template and containing 0.5 μM of each primer as below:

ALAD-sense primer:	5′-AGACAGACATTAGCTCAGTA-3′
ALAD-antisense primer:	5′-GGCAAAGACCACGTCCATTC-3′
MGP-sense primer:	5′-AAGCATACGATGGCCAAAACTTCTGCA-3′
MGP-antisense primer:	5′-GAACTAGCATTGGAACTTTTCCCAACC-3′

The reaction conditions were 200 μM of each dNTP, 10× PCR buffer supplied by Bangalore Genei, 2.5 mM $MgCl_2$, and 3U Taq DNA polymerase. The running conditions were pre-denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s (60 °C for MGP) and synthesis at 72 °C for 1 min. Final extension was conducted at 72 °C for 5 min. The ALAD amplified products were digested overnight with MspI restriction enzyme at 37 °C while MGP gene amplified products were digested overnight with BsrI at 65 °C. Fragments were separated by electrophoresis on 12% polyacrylamide gel and visualized by silver staining. The wild-type (ALAD 1-1) was characterized by a 582-bp fragment while ALAD 1-2 shows 582 and 511 bp fragments. MGP-TT genotype is characterized by 118 bp band, CT genotype (118 and 142 bp bands) and CC genotype (142 bp band).

2.4. Estimation of genotoxicity

2.4.1. Chromosomal aberrations test

This protocol was as described previously [22]. To culture the lymphocytes in whole blood, 2 units of PHA was added to each 5 ml media vial prepared with autoclaved double-distilled water, having RPMI 1640 (5 g/100 ml), sodium bicarbonate (1 g/100 ml), fetal calf serum (10 ml/100 ml), penicillin (100 IU/ml), and streptomycin (100 IU/ml), maintaining pH 7.2–7.5. Then, 1.5 ml of freshly collected whole blood was added to each vial, and the vials were kept for incubated at 37 °C for 72 h. Chromosome preparations were screened after adding colchicine (at 70th hour) to arrest the cells in metaphase stage, fixed in methanol and acetic acid (3:1), flame dried and stained with 4% Geimsa before viewing under microscope and the image recorded in the Medi-Image software program.

2.4.2. Micronucleus testing

Genotoxicity of lead-exposed battery workers can be studied directly in target cells of the buccal epithelium by the micronucleus assay [23]. Exfoliated epithelial cells from buccal mucosa were collected by scraping the middle part of the inner cheeks with wooden spatula after moistening the mouth with water collected. The cells were smeared on slide, dried in air and stained with Geimsa solution. Air-dried slides were screened under the microscope for the analysis of micronuclei.

2.4.3. DNA damage analyses using SCGE

Blood samples were taken for determining the DNA damage along with proper controls. The basic methodology for the SCGE assay followed was that of Singh et al. [24] with slight modification [25]. Slides precoated with normal melting agarose (1%) were layered with low melting agarose (0.5%) with 20 μl of blood sample mixed in the agarose followed by another layer of agarose (1%). Slides were immersed in cold lysing solution overnight at 4 °C. Prior to electrophoresis, the slides were equilibrated in alkaline electrophoresis solution (1 mM Na_2EDTA and 300 mM NaOH, pH > 13) for 20 min and electrophoresis was carried out in the same buffer

for 25 min at 25 V and 30 mA. DNA fragments in each cell migrate at a rate inversely proportional to the size of the fragments. Slides were then washed gently 2–3 times, 5 min each with 0.4 M Tris at pH 7.5 (neutral buffer). After final wash, the neutral buffer was drained and washed with distilled water. Each slide was stained with 60 ml of silver nitrate (0.2 mg/ml) covered with a micro glass coverslip and sealed, and then the slides were washed three times with distilled water [26]. All the silver-stained slides were viewed under a microscope with a CCD camera attachment connected to a computer with Medi-Image software containing frame grabber and with viewer saving in a library, and finally a printable version was obtained.

2.5. Statistical analyses [27]

Chi-square test was used to test the statistical significance of differences in chromosomal aberrations, micronuclei and DNA damage in healthy controls and exposed workers. To determine the number of chromosomal aberrations caused upon exposure to lead, 100 metaphases/sample were examined and the total number of aberrations obtained were calculated. Results are tabulated as percentage values obtained for both the exposed and control subjects. The amount of DNA damage (per 100 cells) was estimated as mean ± SE of tail length calculated as μm. The numbers of micronuclei obtained were counted for 1000 cells/sample and values were tabulated as mean ± S.E. Chi-square analysis was used to determine whether the genotype distribution was in Hardy–Weinberg equilibrium and to compare distributions of alleles and genotypes in the different groups of subjects. *P*-value was analyzed for the significance. Statistical analyses were performed with the MedCalc statistical program. Pearson's coefficient of correlation was carried out to assess the effect of age, duration of working years, smoking and alcoholism as possible confounding factors of lead toxicity.

3. Results

3.1. Demographics and baseline characteristics

Table 1 summarizes the basic characteristics of the study subjects. During the time of presentation, 12 of the 33 occupationally exposed volunteers reporting symptoms of lead toxicity appeared severely malnourished. Blood samples collected from all volunteers were analyzed for CBP and only the parameters related to anemic conditions like hemoglobin are presented here. This is because the hemoglobin levels showed a very wide range of variation as represented in Table 2. Hemoglobin levels of occupational workers ranged between 7.0 and 14.0 g/dl, whereas Hb levels in unexposed workers were 11.3–14.8 g/dl. It was observed that 33 subjects from the exposed group had low Hb levels (below 10 g/dl) compared to the controls. Packed cell volume (PCV) levels ranged from 28 to 37%; there was no significant difference in total white cell count and platelet count between occupational and non-exposed groups.

3.2. Gene polymorphism analyses

3.2.1. Polymorphisms in ALAD gene and their association with lead toxicity

The frequencies for ALAD-1 and ALAD-2 alleles were 0.98 and 0.01, respectively. BLL in workers did not differ significantly among ALAD 1-1, 1-2, and 2-2 genotypes; however, subjects from the ALAD 1-2/2-2 genotype group showed higher BLL concentrations (76–88 μg/dl when compared with subjects from the ALAD 1-1 genotype group (22–79 μg/dl).

3.2.2. Polymorphisms in MGP gene and their association with lead toxicity

The frequencies for T and C alleles were 0.612 and 0.386 respectively. The BLL in workers with genotypes TT + CT was higher than those with mutated homozygotes CC (76–88 μg/dl vs. 22–45 μg/dl, $P < 0.05$). BLL did not differ significantly among the three genotypes.

3.3. Changes in blood parameters

Approximately 29.2% volunteers ($n = 33$) from the occupationally exposed group had hemoglobin levels below 10.0 g/dl. There was no significant difference in total white cell count and platelet count between occupational and non-exposed lead-exposed groups. BLL of occupationally exposed individuals were significantly high compared with the unexposed group (Tables 3 and 4).

3.4. Genotoxicity estimations

3.4.1. Chromosomal aberrations test

The mean percentage of aberrant cells in blood lymphocytes of the exposed workers reporting symptoms of lead toxicity was 13.18% while in control subjects it was 8.41%. There was a significant increase in number of aberrant cells, satellite associations and chromosomal aberrations in exposed subjects compared to controls (Chi-square test; $P < 0.01$) (Table 5). The mean percentage of satellite associations in blood lymphocytes were 15.2 and 10.3 in exposed subjects and controls respectively.

3.4.2. Micronucleus testing

A total number of 113,000 and 107,000 buccal epithelial cells from the exposed and control subjects (1000 cells per individual) were screened for micronucleus. The mean ± SE of micronuclei was 10.05 ± 0.47 in exposed and 2.89 ± 0.22 in the control subjects (Table 6). The mean ± SE of micronucleated cells was 2.90 ± 0.192 in exposed and 1.08 ± 0.15 in control subjects (Chi-square test; $P < 0.01$).

3.4.3. Comet assay

Peripheral blood samples of 113 exposed and 102 control subjects were analyzed for basal DNA damage using comet assay (SCGE). A total of 11,300 cells from exposed and 10,200 from control subjects (100 cells per individual) were screened (Table 7). The mean comet tail-length for the basal DNA damage in workers was found to be 1.511 ± 0.29 μm, whereas, in control subjects it was 0.605 ± 0.14 μm. There was a significant increase in the level of DNA damage in workers compared to controls (Chi-square test; <0.01).

3.5. Effect of confounding factors

Parameters like age, alcoholism, smoking and duration of working years as possible confounding factors to lead toxicity has been investigated. Regression analysis was carried out to test the effect of these factors on BLL. There was no correlation between age and BLL; however, the duration of exposure to lead caused a significant increase in BLL (Fig. 1). Smoking and alcoholism also did not have a significant effect on BLL ($P > 0.05$).

4. Discussion

Although population exposure to lead has significantly declined; chronic toxicity to this metal remains a major public health problem in developing countries. The uptake and toxic effects of lead in some animals have been extensively investigated [28]. However, the bioavailability of lead due to environmental exposure depends on several factors. A systematic analysis of lead poisoning has thus been performed in this study by employing genetic susceptibility,

Table 1
Demographic and baseline characteristics of the study population ($n = 225$).

S.No.	Parameter	Workers ($n = 113$)	Controls ($n = 102$)
1.	Age (years)	18–51 years	18–51 years
2.	Sex		
	Males	100	88
	Females	13	14
3.	Smoking and tobacco chewing		
	Yes	40	9
	No	73	93
4.	Alcohol consumption		
	Alcoholic	39	–
	Non-alcoholic	74	102
5.	Duration of exposure	4–10 years	Not exposed
6.	Period of exposure	6–8 h/day	–
7.	Symptoms of lead toxicity	Gastritis, nausea, vomiting, headache, lethargy and poor appetite	–

Table 2
Hemoglobin ranges of the study group (occupationally exposed and controls) along with normal ranges.

S.No.	Range of hemoglobin (g/dl)	Number of subjects (N)	
		Exposed ($n = 113$)	Control ($n = 102$)
1	7.0–8.0	5	–
2	8.1–9.0	9	–
3	9.1–10.0	19	-–
4	10.1–11.0	39	29
5	11.1–12.0	22	34
6	12.1–13.0	13	27
7	13.1–14.0	6	12

Number of highly anemic individuals in the exposed group was 33. Normal ranges of hemoglobin: Males (13.5–18.0 g/dl); females (11.5–16.5 g/dl).

Table 3
Variations of blood lead levels and hemoglobin levels in ALAD 1-1, 1-2/2-2 individuals.

S.No.	Subjects	Polymorphism	Hb (g %)	BLL (μg/dl)
1	Occupationally exposed individuals ($n = 113$)	ALAD 1-1 ($n = 107$)	7.0–13.4	21.8–79.1
		ALAD 1-2/2-2 ($n = 6$)	10.1–14.2	76.2–88
2	Control group ($n = 102$)	ALAD 1-1 ($n = 100$)	11.3–14.8	0.6–3.4
		ALAD 1-2/2-2 ($n = 2$)	11.2–14.0	1.0–1.9

$P > 0.05$ (Chi-square).

Table 4
Variations of blood lead levels and hemoglobin levels in MGP TT, CT and TT individuals.

S.No.	Subjects	Polymorphism	Hb (g %)	BLL (μg/dl)
1	Occupationally exposed individuals ($n = 113$)	MGP (TT/CT) ($n = 96$)	10.1–14.2	76–88
		MGP (CC) ($n = 17$)	7.0–13.4	21.8–45
2	Control group ($n = 102$)	MGP (TT/CT) ($n = 82$)	11.2–14.0	0.6–3.4
		MGP (CC) ($n = 20$)	11.3–14.8	1.0–1.9

$P > 0.05$ (Chi-square).

Table 5
Chromosomal aberrations obtained in control and exposed subjects.

Subjects	No. of metaphases counted	Percent aberrant cells	Percent satellite associations
Exposed ($n = 113$)	100 cells per individual	13.18%	15.7%
Controls ($n = 102$)	100 cells per individual	8.41%	10.3%

$P < 0.01$ (Chi-square).

Table 6
Micronuclei and percentage of micronucleated cells in the buccal epithelial cells of exposed and control individuals.

S.No.	Subjects	No. of cells counted	Mean ± SE of micronuclei	Mean ± SE of micronucleated cells
1	Controls ($n = 102$)	1000 cells per individual	2.89 ± 0.22	1.08 ± 0.15
2	Exposed ($n = 113$)	1000 cells per individual	10.05 ± 0.47	2.90 ± 0.192

$P < 0.01$ (Chi-square).

Table 7
DNA damage (mean ± SE) of comet tail length (μm) obtained in controls and exposed subjects.

Subjects	No. of cells counted	Mean ± SE of tail length (μm)
Controls (n = 102)	100 cells per individual	0.408 ± 0.04
Exposed (n = 113)	100 cells per individual	1.511 ± 0.09

$P < 0.05$ (Chi-square).

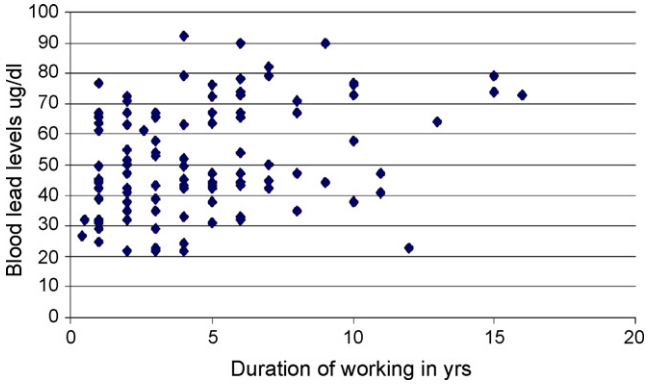

Fig. 1. Effect of confounding factors on BLL.

biochemical and genotoxicity as important parameters in the clinical implications of lead poisoning. Chromosomal aberrations assay, MNT and SCGE were chosen as biomarkers of effect for lead toxicity. Similarly biochemical alterations in blood of exposed and control subjects was investigated by performing BLL, Hb and assessment of alterations in blood samples. Genetic susceptibility to lead poisoning was investigated by determining polymorphisms in two genes ALAD and MGP in relation with BLL of exposed subjects.

The data related to the mutagenic, clastogenic and carcinogenic properties of inorganic lead compounds have been conflicting. Lead (Pb) has been found to be capable of eliciting a positive response in a wide range of biological and biochemical tests like enzyme inhibition assay, fidelity of DNA synthesis, mutations, chromosomal aberrations, cancer and birth defects. Lead was considered a weak mutagen: some studies have shown that this compound is capable of inducing gene and chromosomal mutations, but not all studies have been positive [29,30]. In this study, a significant increase in the frequency of chromosomal aberrations, satellite associations, DNA damage and micronuclei in the buccal epithelial cells was observed in exposed subjects as compared to control individuals. In earlier reports, an increased incidence of micronuclei in peripheral lymphocytes was observed in a group of 22 lead workers whose mean BLL was 61 μg/dl relative to control groups with mean BLLs of 18–28 μg/dl [31,32]. Battery plant workers (n = 37) had significantly elevated levels of DNA breaks in lymphocytes compared to unexposed subjects (n = 29) [30].

Analysis of lead in whole blood is the most common and accurate method of assessing lead exposure [33]. Lead has long been known to alter the hematological system by inhibiting the activities of several enzymes involved in heme biosynthesis. In our present study group, few volunteers had higher BLL probably due to continuous exposure to lead as these individuals were working in battery recycling units. Hemoglobin levels of occupational workers were comparatively lower (7.0–14.0 g/dl) than non-exposed workers (11.3–14.8 g/dl). Among workers, 33 individuals were found to be highly anemic. No significant difference was seen in total WBC and platelet count between workers and control group.

Gene–environment interactions that link exposures, polymorphisms, and disease states are useful in interpreting susceptibility to lead toxicity [7,34,35]. Detection of polymorphisms in such genes can help identification of individuals who may be at increased risk for lead poisoning from environmental or occupational lead exposure. Therefore, analysis of ALAD and MGP genes was taken up; both for the first time in the Indian population. The frequency of ALAD-2 allele varies between 0 and 20% across populations and was lower in our study group. Generally, Caucasians have the highest frequency of the ALAD-2 allele (18% being ALAD 1-2; and 1% being ALAD 2-2). In comparison, African and Asian populations have low frequencies of the *ALAD-2* allele, with few or no *ALAD-2* homozygotes being found in such populations [15]. The existence of this polymorphism and the fact that ALAD is markedly inhibited by lead suggested a possible physiologic relationship between the ALAD isozymes and lead poisoning.

Our results confirm previous findings that show no significant effects of the ALAD G177C polymorphism on BLL concentrations [17,36]. Some studies have suggested that carriers of the ALAD-2 allele would have higher BLL concentrations than non-carriers, thereby increasing their susceptibility to lead toxicity [14,37,38]. In the present study, BLL concentrations varied to levels much above the normal reference ranges independent of the genotype. ALAD 1-2/2-2 type subjects had higher BLLs (76.2–89.1 μg/dl), whereas ALAD 1-1 volunteers showed a BLL range of 21.8–79.1 μg/dl. This can be attributed also to the duration of exposure to lead, number of working hours, and poor nutritional status in some volunteers.

This study also aimed to identify the relation of some SNPs with emphasis on lead toxicity. Since MGP gene is an important biomarker associated with atherosclerotic calcification and involved in calcium metabolism; it can be hypothesized that it may also be associated with lead toxicity. T-138C polymorphisms in the promoter region of this gene is known to influence gene expression level; CC genotype MGP showing the highest levels in blood serum followed by CT and TT. The C genotype (CT + CC) tended to show a higher calcification factor than the TT genotype. Extrapolating from these studies indicate that MGP might form soluble complexes with the metallic ions, which may be discharged easily by the biological system. Compared with the CC genotype, the TT genotype does not favor the lead to discharge, causing the storing up of lead. Therefore, BLL of TT genotype was found to be higher than the CC genotype. Presence of polymorphism in MGP gene may alter the levels of lead in the skeleton and soft tissue which causes storing up of lead in reverse proportion to MGP level. Considering the central role of MGP in vascular calcification and a similar pathogenesis between vascular calcification and kidney stones, we hypothesized that MGP genetic polymorphisms may influence the risk of lead toxicity. The frequency of MGP CC genotype was low in our study group. Previously, an investigation was performed in a Chinese Han population in children by Zhang et al. [39] who showed significant effect of MGP gene polymorphisms with BLL. In the present study, we found that the T-138C polymorphism did not affect BLL concentrations.

Genetic association studies with candidate genes are widely used to study complex diseases caused by epigenetic factors [40]. However, in the population, that we have studied, although levels of lead were varying with respect to polymorphism, significance could not be achieved. This may be because of the sample size; therefore, higher sample size may be required to establish the role of SNPs in lead toxicity. Frequency of ALAD-2 allele was low in our population and may effect the results of association studies. Further research on clinical implications of such high exposures in both genotype individuals on a larger population size may throw more insight with respect to analyses of lead toxicity.

Apart from evaluating the occupational exposure of subjects by using appropriate biomarkers, the confounding factors such as age, years of exposure (years of working) and effect of smoking was also studied in order to assess the possible effect of these parameters among the exposed and control subjects for genotoxicity. It is evident from the data that duration of work showed a significant

effect on lead toxicity (Fig. 1). Similar results were reported in workers occupationally exposed to different occupational settings [38]. In the present study this effect may be explained on the basis of chronic exposure to the lead while manufacturing batteries, which may have accumulated the compounds over the years.

Estimation of hemoglobin, CBP and BLL could be the easiest biomarkers of exposure to lead toxicity. Lead-induced chromosomal aberrations and DNA damage in human lymphocyte cells that can be used as significant biomarkers of effect. The initiation of DNA damage and chromosomal aberrations are markers of genotoxicity and, may be of more relevance to the exposure to immune system. The genotoxic effects detected in vitro may give information on the spontaneous or environmentally determined susceptibility to xenobiotics. Micronucleus assay was performed in buccal epithelial cells since this tissue is in direct contact with air borne pollutants and can give an estimate of the extent of oral exposure thus helping in monitoring human exposure to inhaled occupational and environmental genotoxicants. Micronuclei, thus have a useful application in checking the efficiency of primary prevention strategies, based either on the reduction of human exposure and/or on the stimulation of host defense machinery [41,42]. It could be seen that mutations in ALAD and MGP genes can cause alterations in response of individuals to lead toxicity. Also, differences were observed with respect to lead toxicity and genotyping data in correlation with biomarkers of exposures like hemoglobin content and BLL. Some health conditions can be managed, but some health consequences may not be preventable after a cumulative dose threshold is exceeded [42]. We must prevent cumulative dose, not just follow BLLs but focus on prevention of long-term, progressive health effect. We must acknowledge that there are likely to be susceptible subgroups, demonstrating worse lead-associated outcomes in these persons with these polymorphisms (ALAD) and promulgate lead standards that prevent adverse health outcomes in these most susceptible groups. Factors influencing genetic susceptibility may act at the site of exposure (usually by increasing or decreasing uptake), may affect the toxicodynamics of a metal (usually by complexing or covalent binding) and may influence some immunological, biochemical or cytological functional responses [43,44]. Nutritional supplements are important in the reduction and mitigating the effects of occupational and environmental exposure to lead [45–47].

5. Conclusions

This study reveals that there are many associated risk factors which enhance the toxic effects of lead. Once a significant lead body burden accumulates, the health effects are likely to be progressive and, to a large degree, irreversible, like other chronic diseases. From our study we conclude that lead could induce significant DNA damage and strand breaks. We found that lead is a genotoxicant causing DNA damage and chromosomal aberrations (studies on exposed and unexposed volunteers) and certain factors like, duration of exposure, habits and other demographic factors of the individual enhance the toxicity. It was found that hemoglobin levels were comparatively low in volunteers with higher BLLs, but there was a definite role of the factors like nutritional status, calcium and iron intake of the individual. ALAD and MGP gene polymorphisms have to be analyzed and established as biomarkers of susceptibility on a larger population size before using them for identifying their association with lead toxicity. We strongly recommend routine check of BLLs and use of biological biomarkers (genotoxic assays, BLL) for monitoring the toxic effects of heavy metals like lead. Identification of the various lead sources that surround us can help towards prevention of lead toxicity. Awareness and knowledge regarding lead toxicity helps in minimizing the toxic effects, which is surely a preventable health hazard [47,48]. Along with appropriate chelation therapy as the primary treatment for heavy metal intoxication by lead, supplements with iron appropriate diet in conjunction with definite lifestyle changes help in prevention of lead toxicity to a large extent.

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