

A study on ALAD (G177C and T168C) and MGP (T-138C) gene polymorphisms associated with lead exposure in subjects from Saudi Arabia



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ARTICLE INFO

Article history:

Received 16 October 2017

Received in revised form

5 January 2018

Accepted 6 January 2018

Keywords:

ALAD

MGP

Gene polymorphisms

Lead toxicity

Saudi Arabia

ABSTRACT

Delta aminolevulinic acid dehydratase (ALAD) gene polymorphisms (rs1139488 – MspI and RsaI in exon 4) and Matrix Gla (γ-carboxyglutamic acid) protein T-138C polymorphisms were reported to affect the response of individuals to lead toxicity symptoms. In several previous studies, large inter-ethnic differences in the frequencies of different gene variants have been reported. However, no informational data about the Arab world has been documented. This study was carried out to examine the effects of ALAD and MGP gene polymorphisms on blood lead levels. Method: A total of 127 subjects employed in different professions like battery shops, painting industry and automobile repair shop and plumbers from different parts of Riyadh region, Saudi Arabia. Blood lead levels (BLL), and genotyping, and restriction digestion was performed for each sample. Result: The BLLs in low BLL (<10 µg/dL) group remained well within prescribed safe limits with mean levels of 4.37 µg/dL. In high BLL (>10 µg/dL) group however, the levels were significantly elevated to 18.12 µg/dL (p<0.001). Workers involved in battery acidifying area were found to have high blood lead level (18.70 µg/dL), followed by those involved in plate making process (12.57 µg/dL) and opening and breaking of old batteries (10.77 µg/dL). On the other hand, lead level was found to be as low as <3.3 µg/dL among the workers who were not involved in battery breaking or manufacturing process (administration, drivers and security personnel). Overall, the genotype frequencies of GG and GC+CC for ALAD exon 4 MspI restriction site (G177C) were 96.7% and 3.3%, with allele frequencies of 0.979 (G) and 0.01 (C), respectively. For ALAD exon 4 RsaI restriction site (T168C), the frequency of TT and TC+CC genotypes were 65% and 35% with allele frequency of 0.779 (T) and 0.220 (C) respectively. For the Matrix Gla (γ-carboxyglutamic acid) gene (MGP) T-138C polymorphism, the variants TC and CC were not detected in this population; all subjects had TT genotype. BLLs for ALAD G177G, ALAD C168C and MGP T-138T genotypes from High BLL group were 18.70, 19.09 and 15.05 µg/dl respectively.

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1. Introduction

Lead is currently used in more than 900 occupations, including battery manufacturing, smelting and mining (ChemIDplus, 2005). Lead poisoning occurs as a result of ingestion or

inhalation of inorganic lead particles or through transdermal absorption of organic alkyl lead (RTECS, 2007; Bellinger, 2004). Lead is commonly incorporated into rapidly growing bones, such as the tibia, femur, and radius, where it competes with calcium and may exert toxic effects on skeletal growth (ATSDR, 2006). The definition of an elevated concentration of lead in the blood, according to the Centers for Disease Control and Prevention (CDC, 1991), is 10 µg/dl. Exposure to lead can have a wide range of biological effects depending on level and duration of exposure.

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<https://doi.org/10.21833/ijaas.2018.03.008>

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Genetic biomonitoring of populations exposed to hazardous substances like heavy metals could create an early warning system for genetic diseases (Osorio and Melius, 1995; RTECS, 2007). Many types of occupational exposure pose serious health hazards (Eaton and Klaassen, 1996). Thus, determining polymorphisms that affect susceptibility allows identification of susceptible groups and their appropriate treatment measures. Our search of various scientific databases indicated that many studies were not performed in the Saudi Arabian population with respect to polymorphisms Delta aminolevulinic acid dehydrates (ALAD) and Matrix Gla (δ -carboxyglutamic acid) protein (MGP) and their possible role in lead toxicity.

Delta aminolevulinic acid dehydratase (ALAD) enzyme, also known as porphobilinogen synthase (PBG Synthase) has been extensively recognized to play a significant role in lead poisoning (Shaik and Jamil, 2008; Bergdahl, 1998). ALAD enzyme which catalyzes an important step in the synthesis of heme molecule is inhibited by Pb^{2+} ions. At the molecular level, lead displaces a zinc ion at the metal binding site (not the active site) producing inhibition through a change in the enzyme's quaternary structure. This inhibition of ALAD results in the buildup of aminolevulinic acid, detectable in the plasma and urine at BLL less than 10 $\mu\text{g}/\text{dl}$ (Schwartz et al., 1995). Thus, ALAD enzyme plays a major role in response to metal poisoning caused by lead, which strongly inhibits ALAD stoichiometrically (Wetmur et al., 1991a; Wetmur et al., 1991b; Schwartz et al., 1995). It is important to note that the gene coding for ALAD enzyme (chromosome 9q34) is polymorphic in nature; the ALADG177C polymorphism yields two co-dominant alleles, ALAD-1 and ALAD-2 which have been implicated in susceptibility to lead toxicity. The ALAD-2 allele contains a G \rightarrow C transversion at position 177 of the coding region, resulting in the substitution of asparagine for lysine at amino acid 59 that define three isozymes, viz., 1-1, 1-2, and 2-2. ALAD 1-2 heterozygotes produce an enzyme that is more electronegative than that of ALAD-1 homozygotes, and ALAD-2 homozygotes produce an enzyme that is more electronegative than that of 1-2 heterozygotes. It has been indicated that ALAD 1-2/2-2 individuals may have high BLLs, lesser amounts of chelatable lead. Studies have suggested that carriers of the ALAD2 allele have higher BLL than ALAD1 subjects and thus are more susceptible to lead toxicity (Wetmur et al., 1991b; Kelada et al., 2001; Onalaja and Claudio, 2000; ATSDR, 2006). Some authors suggest that the enhanced capacity of ALAD2 to bind lead may confer resistance to its harmful effects because subjects with ALAD2 may have less bioavailable lead (Schwartz et al., 1995). Genotype frequencies vary by geography and race (Onalaja and Claudio, 2000). Polymorphisms in the ALAD gene that affect lead toxicity have not been studied as yet in the Saudi population.

Matrix Gla (γ -carboxyglutamic acid) protein (MGP) is a 10-kDa secreted protein containing five

residues of the vitamin K-dependent calcium binding amino acid Gla. Recent evidence indicates that MGP functions as an inhibitor of mineralization and is synthesized in vivo mainly in cartilage and in the vascular system (Shanahan et al., 1998). MGP protein contains five residues of the vitamin K-dependent calcium-binding amino acid, Gla (Shanahan et al., 1998). Although the precise molecular mechanism remains unknown, all available evidence indicates that MGP plays a role as an inhibitor of mineralization (Afshin et al., 2001; Shanahan et al., 1998). MGP also contains a fourth exon of unknown function that codes for 11 residues and lies between the transmembrane signal peptide and the putative recognition site for the gamma-carboxylase (Price et al., 2002; Afshin et al., 2001). This four-exon organization is essentially identical to that of bone Gla protein (112260), but is quite different from the two-exon organization encoding this region in other known vitamin K-dependent proteins. Cancela et al. (1990) identified two regions of the promoter containing possible binding sites for retinoic acid and vitamin D receptors. The T-138C polymorphism in MGP promoter region is known to either enhance or decrease synthesis of this protein, thus affecting the mineralization.

MGP gene suppresses calcium ion function in the cartilage, and other soft tissues, in addition, lead and calcium are divalent cations, with the same absorption pathways. 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 current study will explore the relationship between polymorphisms in the promoter region of MGP gene and investigate its probable association with lead poisoning and also will assess the influence of MGP genotypes with relation to hematological parameters. Taken together, ALAD gene polymorphisms influence the accumulation and distribution of lead in the blood, bone, and internal organs in humans and animals. MGP gene also controls calcium metabolism. Because of these polymorphisms and their effect on bone mineralization, it can be expected that these genetic variants may also influence lead accumulation in the body. The objective of this investigation was to determine the influence of ALAD G177C, ALAD T168C and MGP T-138C genotypes in the study group occupationally exposed to lead with relation to BLLs. Since Delta aminolevulinic acid dehydrates plays an important role in lead poisoning, it might be possible to identify those individuals who could be more susceptible to the toxic effects of lead using genotype assay. given the heterogeneity of the Arab populations, and the fact that there are absences of such studies in this region, such a study will help in raising awareness to heavy metal toxicity.

2. Methods

Subjects employed were working in different professions like battery shops, painting industry and

automobile repair shop and plumbers from different parts of Riyadh region, Saudi Arabia. Prior to collecting the blood samples, each individual had to undergo an extensive interview about their disease history, dietary habits, information on job experience, socioeconomic situations like income and education, and history such as drug uses, consumption of vitamins or antioxidant supplements, smoking, and dietary habits was performed from questionnaires. Detailed preform was collected from all subjects and they were made aware of the study protocol and their permission was taken before enrolling them in the study. The departmental research Ethics Review Board approval was granted by the KSU. All subjects were submitted to comprehensive clinical assessment to diagnose symptoms of hematological and neurological diseases. Patients with a history of disease, use of antioxidant/vitamin supplements or other drugs, exposure to other toxic compounds, radiotherapy, and substance abuse were not allowed to participate in the research.

A total of 200 samples were collected. For blood collection and laboratory investigation. Firstly, skin was cleaned thoroughly and sterilized with a 70% alcohol swab and dried before withdrawing 5ml of peripheral blood by a 5 cc disposable syringe from enrolled subjects. For hematological variables blood was collected in EDTA tubes.

The amount of lead concentration in plasma samples was measured by LeadCare II analyzer.

2.1. Gene polymorphism analyses

PCR was conducted using QIAGEN HotStarTaq Master Mix Kit in a volume of 50 μ l [2 μ l genomic DNA (2 μ g/ μ l), 25 μ l HotStar Taq Master Mix, 19 μ l RNase free water, 2 μ l of each primer] in a Thermal cycler (Mastercycle Personal, Eppendorf, Germany). The amplification conditions were as follows: Initial denaturation at 95°C for 15 min, followed by 35 cycle of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, followed by final extension at 72°C for 10 min. The PCR product was purified by using the isolate PCR kit (Bioline Inc., USA). The DNA bands were visualized under UV light and photographed using gel documentation (Molecular Imager® Gel Doc™ XR + Systems with Image Lab™ 2.0 Software, BioRad, USA). The ethidium bromide (fluorescent dye) intercalated between bases of DNA causing the visualization of the bands. The gel was then photographed.

2.2. Amplification and genotyping for rs1139488 SNIP in ALAD gene

Genotyping of the G177C and T168C polymorphism was carried out using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. The resulting DNA fragment was 713bp in length. The genotypes for this SNP were determined by restriction fragment

length polymorphism (RFLP) procedure using 1 μ l of restriction enzyme (*MspI*). The tubes were incubated for 4 hours at 37 C followed by heat inactivation for 15 minutes at 65°C. After that, the genotypes were resolved after running it on 2% (w/v) agarose gels electrophoresis. A second restriction enzyme *RsaI* was also used to make an internal cut within the PCR fragment as below:

ALAD Forward: GTTGCAGAAGGGAGCTGAAC
Reverse: ACCTTTGCCCAACCTCC

2.3. Amplification and genotyping for MGP gene

PCR-RFLP for MGP gene was carried out using previously described primers by [Zhang et al. \(2003\)](#). Genotypes of MGP gene were analyzed after restriction digestion with *BsrI* restriction enzyme under similar conditions as described above.

2.4. Statistical analysis

Otherwise specified, data are presented as mean \pm SE. 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. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Clinical characteristics of the enrolled subjects

The study enrolled 200 subjects aged (19-65 years) from various regions of Riyadh, Saudi Arabia. Some subjects refused to participate in the clinical investigation or interview procedure. All subjects were categorized into two groups based on blood lead levels (BLLs) and as per the guidelines suggested by CDC and as mentioned in the project. Some subjects were excluded for the study when the BLL readings were uninterruptable or doubtful. Therefore there were subjects with BLL > 10 μ g/dL called High BLL group and subjects with BLL < 10 μ g/dL called Low BLL group. A total of mean age of High BLL subjects was 32.9 years while the mean age of Low BLL group was 35.8 years. All the recruited subjects were male. The demographic and clinical features of the entire studied population are presented in [Table 1](#).

3.2. Comparison between blood lead levels of all subjects

The blood lead levels in low BLL group remained well within prescribed safe limits with mean levels of 4.37 μ g/dL. In high BLL group however, the levels were significantly elevated to 18.12 μ g/dL ($p < 0.001$). Workers involved in battery acidifying area were found to have high blood lead level

(18.70 µg/dL), followed by those involved in plate making process (12.57 µg/dL) and opening and breaking of old batteries (10.77 µg/dL). On the other hand, lead level was found to be as low as <3.3 µg/dL among the workers who were not involved in battery breaking or manufacturing process (administration, drivers and security personnel).

Table 1: Clinical characteristics of enrolled subjects

Parameter	Low BLL	High BLL
N	65	22
Age (range)	19-65 years	21-52 years
Mean age	35.8 years	32.9 years
% females	0%	0%
% males	100%	100%

3.3. Gene polymorphism studies

This 2 gene SNIP variant analysis generated the genotypic data, first time available for analysis in the local population that comprised of residents from Riyadh, Saudi Arabia, showing marked genotypic differences. These variants haplotypes may propose the susceptibility combination of specific genes towards Pb toxicity related to specific trend of allelic presentation.

3.4. Polymorphisms in various genes in the overall study population

Genotype frequencies of ALAD (177C variant, ALAD T168C variant, and MGP T-138C in the overall

study population are presented in Table 2. The distribution of genotypes for all genes were in Hardy-Weinberg equilibrium.

3.5. Polymorphisms in various genes in the Normal BLL group

To check if considerable variations occur amongst the subjects with normal BLL group, genotype frequencies were evaluated. The results are presented in Table 3.

3.6. Polymorphisms in various genes in the High BLL group

To check if considerable variations occur amongst the subjects with high BLL group, genotype frequencies were evaluated. The results are presented in Table 4.

3.7. Distribution of blood lead levels in all subjects categorized by genotypes

The blood lead levels were further categorized by various identified genotypes and compared with subjects with normal levels. Irrespective of the genotypes, all subjects from high BLL group had significantly increased BLLs compared with normal group. The results are presented in Table 5 and corresponding Fig. 1.

Table 2: The distribution of genotypes and allele frequencies in the overall study population

		Genotype frequency (%)	Allele frequency	Chi square	Chi square p value
ALAD G177C variant	G,G	96.7	G=0.979	18.47	0.000017
	G,C+C,C	3.2	C=0.02		
ALAD T168C variant	T,T	65	T=0.779	0.09	0.75
	T,C+C,C	35	C=0.220		
MGP T-138C	T,T	100	T=1	NA	NA
	T,C+C,C	0	C=0		

Table 3: The distribution of genotypes and allele frequencies in subjects with normal BLL

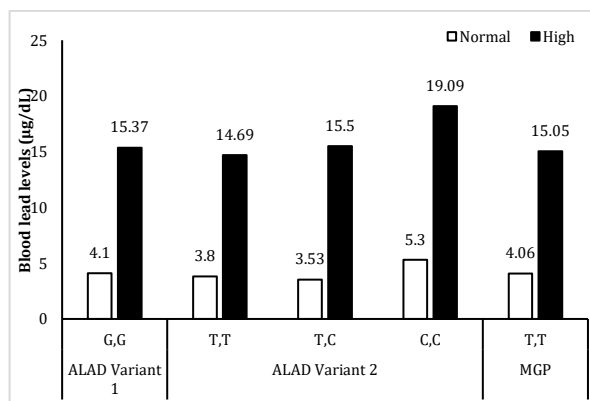
		Genotype frequency (%)	Allele frequency	Chi square	Chi square p value
ALAD G177C Variant	G,G	97	G=0.985	0.022	0.88
	G,C	3	C=0.014		
	C,C	0			
ALAD T168C Variant	T,T	67.9	T=0.803	0.28	0.59
	T,C	30.1	C=0.196		
	C,C	3.7			
MGP T-138C	T,T	100	T=1	NA	NA
	T,C	0	C=0		
	C,C	0			

Table 4: The distribution of genotypes and allele frequencies in subjects with high BLL

		Genotype frequency (%)	Allele frequency	Chi square	Chi square p value
ALAD G177C Variant	G,G	95	G=0.95	20	0.000008
	G,C		C=0.05		
	C,C	5			
ALAD T168C Variant	T,T	60	T=0.75	0.8	0.37
	T,C	30	C=0.25		
	C,C	10			
MGP T-138C	T,T	100	T=1	NA	NA
	T,C		C=0		
	C,C				

Table 5: Blood lead levels in the study population categorized by genotypes

		Normal	High	p value
ALAD G177C variant	G,G	4.1	15.37	<0.001
	T,T	3.8	14.69	<0.001
ALAD T168C variant	T,C	3.53	15.5	<0.001
	C,C	5.3	19.09	<0.0001
MGP T-138C	T,T	4.06	15.05	<0.001

**Fig. 1:** Subjects showing significantly high BLL with their corresponding genotypes compared to normal subjects

4. Discussion

While exposures to chemicals at the workplace and other pollutants in the environment are a public health issue for the general population, they pose a particular health threat to persons who are genetically predisposed to react adversely to them (CDC, 1997). Because lead is a commonly used heavy metal, biochemical and molecular testing could play a significant role in reducing the disease burden associated with lead toxicity. Gene association studies with respective genes are commonly used to understand complex diseases caused by genetic/epigenetic and environmental factors. The current study investigated the relation between blood lead levels and gene polymorphisms in ALAD and MGP genes in subjects employed in various professions like battery shops, painting industry, automobile repair shops, and plumbing from Riyadh region of Saudi Arabia. This study assumes significance owing to the absence of such studies in this region. It is well known that measuring Blood lead levels (µg/dL) is the most commonly used biological index for lead exposure (Bressler and Goldstein, 1991). However, many studies reported that polymorphisms in certain genes can modulate the levels of lead in the blood of exposed subjects. Hence, it is essential to understand the extent of lead poisoning through genetic monitoring studies. All subjects recruited in this study were screened and categorized into two sub-groups of below 10 µg/dL or higher than 10 µg/dL based on CDC guidelines (CDC, 1991). A large number of subjects had BLL levels of 4.37 µg/dL, but, a proportion of subjects showed BLLs of 18.12 µg/dL ($p < 0.001$).

To the best of our knowledge, this is the first study that evaluated the possible effects of ALAD

G177C/T168C and MGP T-138C polymorphism in relation to BLL in lead exposed workers from Riyadh, Saudi Arabia. The frequency of ALAD (GC) 1-2 and ALAD (CC) 2-2 genotypes was very low (3.2%) in our study group. It has been reported that Caucasians have the highest frequency of ALAD 1-2 (18%) and ALAD 2-2 (1%) population. In contrast, the African and Asian populations have lower frequencies of the ALAD-2 allele (Kelada et al., 2001). Owing to the fact that the Arab population forms a unique ethnic group genetically and the absence of such studies in this region, it is important to evaluate and correlate BLL with gene variants. Studies conducted in Saudi Arabia focused on school girls, male volunteers and pregnant women (Al-Saleh, 1995; Al-Saleh et al., 1995; Al-Saleh et al., 1999); and in a more recent study approximately 52% of healthy volunteers from Al-Batha and Olya regions of Riyadh having BLL >10 µg/dL (Al-Othman et al., 2013).

Biochemically, the ALAD 2-2 enzyme has a replacement of asparagine for lysine at amino acid at 59 position (Wetmur et al., 1991a). This substitution renders the ALAD 1-2 heterozygotes and ALAD 2-homozygotes to synthesize a more electronegative enzyme compared to ALAD-1 homozygotes. Since studies indicate that ALAD is significantly inhibited by Pb, it has been believed that a molecular and biochemical association exists amongst ALAD isozymes and lead toxicity. Studies also indicate that subjects with ALAD2 alleles have high BLLs, hence making them more susceptible to lead poisoning. However, in the present study, we found that the ALAD G177C or T168C polymorphism did not influence BLL concentrations. BLLs varied to levels of less than 10 µg/dL in a majority of subjects, approximately 22 subjects (17%) had a mean BLL of 18.12 µg/dL, a level much above the CDC threshold. Interestingly, approximately 23% of the subjects with High BLL had CC genotype. For the ALAD G177C polymorphism, BLLs were independent of the genotype in the Low vs High BLL groups. The ALAD 1-1 (ALAD G177G) genotype subjects from High BLL group showed levels of 15.37 µg/dL; the ALAD G177C (ALAD 1-2) and ALAD C177C (ALAD 2-2) were minimal (3%) in this population. It is important to note that the length of exposure to lead, working hours and poor nutritional status may have contributed to these results in subjects with high BLL. Our results indicate that ALAD 1-2/22 variants may not play a substantial role in modulating BLL at higher exposure levels (Schwartz et al., 1995).

These results are partly in support and partly in contrast to the studies of Montenegro et al. (2006a) signifying no significant differences in BLLs among lead exposed subjects, however, individuals with ALAD 1-2/2-2 genotype had higher BLLs. Another study by the same group (Montenegro et al., 2006b) indicated that interethnic differences existed in the distribution of G177C ALAD variants; a factor that could have played a role considering the unique ethnicity of the Arab population. Our results also

conform to findings of [Hu et al. \(2001\)](#) and [Schwartz et al. \(1995\)](#) who reported no significant effects of the ALAD G177C polymorphism on BLL concentrations.

More importantly, this study reports a novel T168C polymorphism in the same exon where G177C polymorphism is located i.e., exon 4. The T168C site can be cleaved by the restriction enzyme RsaI has been identified in exon 4 of the ALAD gene. Approximately 35% of the recruited subjects had the TC/CC genotype; with the dominant genotype accounting for 65%. All genotypes did not differ significantly in lead levels in the Low BLL group. However, in the high BLL group, the TT, CT and CC genotypes had blood concentrations of 14.69, 15.5 and 19.09 µg/dL. This indicates that BLLs were not different with respect to of genotype; however, the CC genotype subjects had relatively higher BLL compared to either TT or CT genotypes. Whether this polymorphism has a cause-effect relationship with regards to lead toxicity remains to be investigated in larger and multiple studies not only in this population but across different ethnicities.

In addition to ALAD, this study also aimed to investigate the T-138C polymorphism in MGP gene due to its role in calcium metabolism. Since, Pb²⁺ ions can compete with Ca²⁺ ions; MGP protein may influence lead deposition at a cellular level. The T-138C polymorphism in the promoter of MGP gene has been shown to influence gene expression levels with CC genotype MGP showing the highest levels in blood serum followed by CT and TT ([Afshin et al., 2001](#)). To our knowledge, this is the first study describing the effects of MGP T-138C polymorphism on BLLs in occupationally exposed workers from Saudi Arabia. However, subjects with MGP CT and CC genotypes were not found in our study group. It is interesting to note that in High BLL subjects, levels as high as 15.05 µg/dL with TT genotype. An investigation by [Zhang et al. \(2003\)](#) showed significant effects of MGP gene polymorphisms with BLLs. Similar results have also been reported in an earlier study where TT genotype showed higher BLL ([Shaik and Jamil, 2009](#)).

According to the results of this study, BLL higher than allowed limit (<10 µg/dL) observed in the workers verifies the presence of contamination and absorption of lead into the body. In this regard, many studies have already reported that BLL in such workers is higher than controls. The results of our analyses showed that there was no association between BLL genes specific targeted SNIP variants in workers. In our earlier published study, some lead workers indicated that they suffered from clinical symptoms of abdominal pain, blue line of gums and peripheral neuropathy like memory loss, less attention, sleeplessness, headache, claudication, epigastric pain, lack of appetite, anxiety, tremor, lessened reflect of deep tendon, deafness, and tiredness ([Alsaeed et al., 2017](#)).

Workers' education, use of personal protective equipment's, and discontinuing the use of lead as much as possible, were suggested as helpful

measures in lowering the toxic effects of occupational exposure to lead. The accurate use of shielding implements and taking day-to-day bath by workers could assist to protect them and specially their children from exposure to lead and other poisonous elements. Based on gene allele data we will be able to screen and predict the Pb susceptible group of individuals who were more prone to Pb toxicity and may be used for prescreening for certain high risk population suspect Pb toxicity and certain clinical condition like neuro-behavioral attitude etc. It is evident from the above results that high BLL is usually accompanied with one or more health issues that are otherwise easily preventable. We therefore, strongly endorse regular assessments of BLLs and gene-environment studies in occupationally exposed subjects from Saudi Arabia in order to monitor the toxic effects of lead. Improved awareness and scientific knowledge concerning lead toxicity aids in reducing the toxic effects. Though doctors have a very good understanding of the treatment options in case of lead toxicity, it is about time that researchers work towards linking the toxic effects of exposure and their affects at a cellular and molecular level.

Acknowledgement

We thank all the volunteers in different professions like battery shops, painting industry and automobile repair shop and plumbers in different parts of Riyadh region, Saudi Arabia who participated in this study. This work was supported by National Science Technology and Innovation plan NSTIP strategic technologies programs, project number NPST-11MED1919-02, in the Kingdom of Saudi Arabia

Conflict of interest

The authors report no conflicting interests with respect to this study.

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