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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