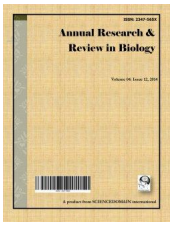

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Association of Delta-Aminolevulinic Acid Dehydratase Polymorphism with Blood Lead and Hemoglobin Level in Lead Exposed Workers

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Authors' contributions

This work was carried out in collaboration between all authors. Author AN collected samples, carried out experiments and prepared the first draft of the manuscript. Authors AP and NS helped in sample analysis and in proof reading of the manuscript. Authors JV and IS contributed in the detection of blood lead levels. Authors AP, SC and DJ were involved in experimental designed, statistical analysis and overall management of the project. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Despite decades of intensive research, lead toxicity still remains one of the most health concerns. Hence, risks posed by lead are more likely to be determined by individual susceptibility as delta-aminolevulinic acid dehydratase (ALAD) gene can modify lead toxicokinetics.
Method: The present study was aimed to evaluate the association of ALAD gene polymorphism (rs1800435 C/G) (ALAD 1-1, ALAD 1-2, ALAD 2-2) with blood lead level (BLL) and hemoglobin (Hb) content from 200 lead-exposed workers of Gujarat, India against 200 controls.
Results: ALAD genotype frequency was found to be 90%, 8% and 2% in control whereas 80%, 14.5% and 5.5% in workers for ALAD 1-1, 1-2 and 2-2 genotypes, respectively. ALAD 1-1 genotype was attributed to higher BLL and lower Hb content as compared to ALAD 1-2/2-2 genotype in

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workers. Whereas, inverse association had been observed between BLL and Hb content in workers having ALAD 1-1 genotype. On the other hand, ALAD 1-2/2-2 genotype might play an important role in lead toxicity by decreasing free lead in blood and by transporting into tissues due to more binding affinity. So, it may protect Hb against free lead by decreasing lead availability in blood.
Conclusion: To deal with lead toxicity more effectively, attention should be given to the workers having the ALAD 1-1 genotype.

Keywords: ALAD genotype; BLL; hemoglobin; lead exposed workers.

1. INTRODUCTION

Lead is a natural constituent of the earth's crust, but once mined and transformed into man-made products which are more accessible forms of lead to human being. Lead had never been fully eliminated from the environment. Moreover, there is a paucity of data regarding effective natural or biological protection against lead toxicity. The interaction of lead and its compounds can result in chronic occupational lead poisoning [1].

The regulatory authority of government is mainly responsible for the reduction in lead toxicity since many years in India. Despite the decline in lead usages, there are many apprehensions regarding the toxic effects of lead exposure and the subtle health effects in occupationally lead-exposed workers. Occupational lead poisoning is almost without exception chronic and occurs after several weeks or months of continuous exposure. There are some considerable inter-individual variations in the toxicokinetics of lead [2,3]. Whole blood lead level (BLL) is usually used to evaluate lead exposure for both screening and clinical diagnostic purposes. BLL is the most frequently used biomarker of recent lead exposures. According to the US Food and Drug Administration, the lowest observed adverse effect level for blood lead concentration is 10 μg dL^{-1} in adults [4]. However, no lead level can be considered as a safe value. It is generally recognized that BLL is not an alone sensitive biomarker for lead exposure in hematological studies. Considering the variation in hemoglobin (Hb) levels along with BLL in different genetic situations may be more relevant when evaluating the hematotoxicity of blood lead [5-6].

After lead is absorbed into the bloodstream, most of it gets into red blood cells (RBCs) and combines with the proteins inside. The toxic effect of lead combination results in the inhibition of the activity of three enzymes in the biosynthetic pathway, delta-aminolevulinic acid dehydratase (ALAD), coproporphyrinogen oxidase (COPRO-O) and ferrochelatase (FERRO-C), giving rise to the abnormal heme synthesis [7]. The importance of ALAD is being most profound in lead toxicity study and is the main lead-binding protein inside erythrocytes. Lead inhibits ALAD stoichiometrically, and the degree of ALAD inhibition has been used clinically to gauge the degree of lead poisoning. Many researchers [3,8-11] had reported ALAD polymorphism, as a possible determinant of susceptibility to BLL among lead-exposed workers. The ALAD polymorphism codes for one of three isozymes (ALAD 1-1, ALAD 1-2, and ALAD 2-2) and responsible for variation in tissue binding and in the long-term deposition of lead in the body [10]. ALAD 1-2 or 2-2 allele has been shown to affect the toxicokinetics of lead exposure by coding for an effective electronegative enzyme that may bind lead ions (positively charged) more firmly than ALAD 1-1 genotype.

Henceforth, the present study reports a cohort evaluation of BLL, Hb level, ALAD genotype polymorphism and their correlation in workers exposed to lead in different factories located in Gujarat, India along with non-exposed healthy individuals as a control. Along with that, this study also comprises the comparison of ALAD polymorphism in occupational lead-exposed workers to find more vulnerable genotype for environmental and occupational lead toxicity.

2. MATERIALS AND METHODS

2.1 Study Population

All voluntaryparticipants were provided with detailed information about study design and its implication, while their informed written consent and questionnaire were availed during counselling session. All the study protocols were approved by the Institutional Ethical Committee of Department of Zoology, Gujarat University. Occupationally exposed individuals were selected or rejected based on the basis of inclusion and exclusion criteria (Fig. 1).

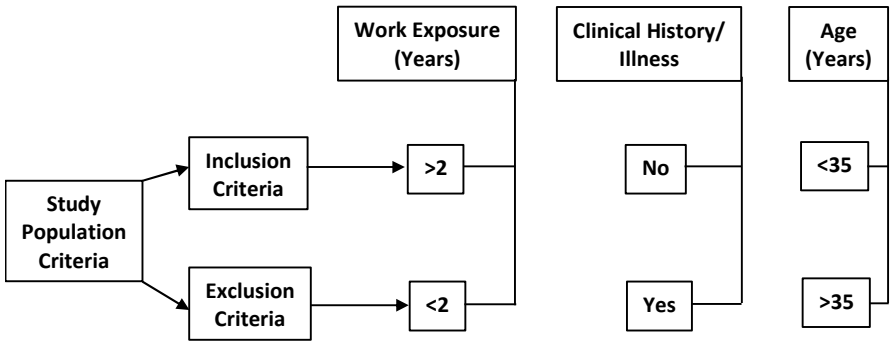

Fig. 1. Study population criteria

The inclusion criteria were 1) Minimum two years of occupational exposure to lead; 2) Do not represent the clinical history; 3) Occupationally exposed participants should fall in the age bar of 25 to 35 years. However, the exclusion criteria for the study were 1) Less than two years of occupational exposure to lead; 2) Having any clinical history; 3) Below or above the age bar of 25 to 35 years. Exposed individuals (n=200) were directly engaged in lead using operations in brass, ceramic, dyeing, glaze, painting and smelting factories of Gujarat, India. Whereas, controls (n=200) were healthy individuals (25 to 35 years) from the same study area.

2.2 ALAD Gene Polymorphism

A modified polymerase chain reaction (PCR) based protocol [3] was used for ALAD genotyping from isolated genomic DNA using Proteinase K. The isolated DNA samples were amplified with the help of the forward and reverse primers 5'-AGAC AGAC ATTA GCTC AGTA-'3 and 5'-GGCA AAGA CCAC GTCC ATTC-'3, respectively. The MspI polymorphism of the ALAD gene was analyzed by restriction fragment length polymorphism (RFLP) method [11]. The digested products were denoted as ALAD 1-1 (582 bp), ALAD 1-2 (582 and 511 bp) and ALAD 2-2 (511 bp) polymorphic forms and detected using 2% agarose gel electrophoresis.

2.3 Blood Lead Level (BLL) and Hemoglobin (Hb) Measurement

To measure BLL, heparinized whole blood from both control (n=200) and lead-exposed workers (n=200) were digested according to The United States Environmental Protection Agency (USEPA) guideline (Method – 3052) in Microwave digestion system (Milestone, Model Start D, Italy) with slight modifications [12]. BLL was measured in triplicate with a background corrected atomic absorption spectrophotometer (Graphite Furnace Atomic Absorption Spectrophotometer, Perkin Elmer, USA) at the Laboratory of Occupational Hygiene (NABL certified), National Institute of Occupational Health (NIOH), Ahmedabad, Gujarat. Hb level detection was assayed in automated cell counter (Sysmex 21, USA).

2.4 Data Analysis

Data were expressed as Mean ± SD. The statistical significance was evaluated by one-way analysis of variance (ANOVA) and the individual comparison was obtained by Tukey's multiple comparison and Student's t-test using GraphPad Prism 7 version. A value of $P<.05$ was considered as a significant value.

3. RESULTS AND DISCUSSION

3.1 ALAD Genotype

In the control group, 180 ALAD 1-1 homozygous (90%), 16 ALAD 1-2 heterozygous (8%) and 4 ALAD 2-2 homozygous (2%) ALAD genotype were identified. In lead-exposed workers group, 160 ALAD 1-1 homozygous (79.84%), 29 ALAD 1-2 heterozygous (13.18%) and 11 ALAD 2-2 homozygous (6.98 %) were identified (Table 1).

3.2 Blood Lead Level (BLL)

The average BLL in workers (16.18 μg dL^{-1}) were significantly higher ($P<.001$) as compared to control individuals (3.79 μg dL^{-1}). The mean BLL in control group, according to ALAD 1-1, ALAD 1-2 and ALAD 2-2 genotypes were 3.46, 2.35 and 5.56, whereas in workers 18.10, 16.87 and 13.59 μg dL^{-1} respectively (Table 1). When the BLL in different polymorphic form of ALAD of control

individuals compared with the respective polymorphic form of ALAD of lead exposed workers (Fig. 2), it showed a significant increase (*P*<.01 for ALAD 1-2; *P*<.001 for ALAD 1-1; *P*<.05 for ALAD 2-2) in the blood lead level. However, the BLL was non-significant low in ALAD 1-2 or 2-2 as compared to ALAD 1-1 in lead exposed workers (Fig. 2).

3.3 Hemoglobin (Hb) Level

The Hb level in lead-exposed workers (11.44 g dL^{-1}) were significantly lower (*P*<.001) as compared to control individuals (14.09 g dL^{-1}) (Table 1). Hb concentration in different ALAD polymorphic forms of the control group was 14.28, 14.00 and 14.00 g dL^{-1}, whereas in the lead exposed workers it was 10.01, 11.20 and 13.10 g dL-1 in ALAD 1-1, ALAD 1-2 and ALAD 2-2 genotypes, respectively (Table 1). There was a significant decrease (*P*<.001) in Hb levels in ALAD 1-1 and 1-2 genotype, as well as the non-significant decrease in ALAD 2-2 genotype was observed in exposed workers as compared to a respective ALAD polymorphic form of control individual (Fig. 3). However, Hb levels in ALAD 1-2 or 2-2 polymorphic forms were significantly high (*P*<.05 for ALAD 1-2; *P*<.01 for ALAD 2-2) as compared to Hb level in ALAD 1-1 genotype of lead-exposed workers (Fig. 3).

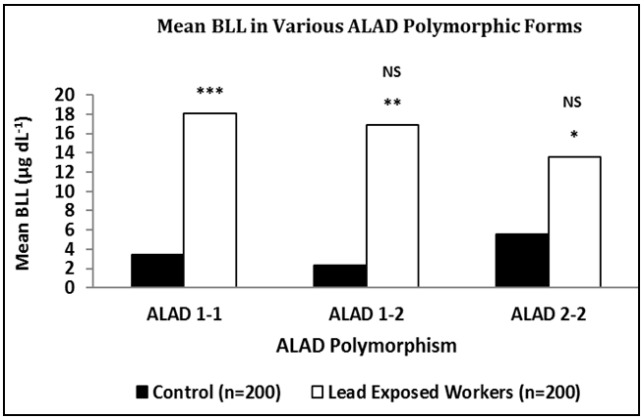

Fig. 2. Comparison of BLL in control and lead exposed workers in various ALAD gene polymorphic forms

**P<.05; **P<.01; ***P<.001; when BLL of lead exposed workers were compared to control in each ALAD polymorphic forms separately; NS=non-significant when BLL of ALAD 1-2 or 2-2 genotypes were compared to ALAD 1-1 genotype of lead exposed workers; BLL=Blood Lead Level*

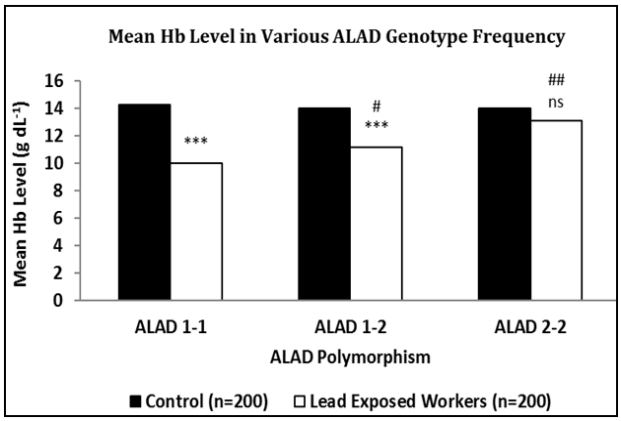

Fig. 3. Comparison of Hb level in control and lead exposed workers in various ALAD gene polymorphic forms

****P<.001; ns=non-significant when Hb levels of lead exposed workers were compared to control in each ALAD polymorphic forms separately; #P<.05; ##P<.01 when Hb level of ALAD 1-2 or 2-2 genotypes were compared to Hb level of ALAD 1-1 genotype of lead exposed workers; Hb=Hemoglobin*

Table 1. BLL and Hb level in control and lead exposed workers in different ALAD polymorphic forms

Groups	Control (n=200)			Lead exposed workers (n=200)		
Genotype	**ALAD 1-1**	**ALAD 1-2**	**ALAD 2-2**	**ALAD 1-1**	**ALAD 1-2**	**ALAD 2-2**
Number	180 (90%)	16 (8%)	4 (2%)	160 (79.84%)	29 (13.18%)	11 (6.98%)
BLL	3.46±2.58	2.35±0.95	5.56±1.41	18.10±11.54	16.87±12.56	13.59±6.75
(μg dL^{-1})	3.79±1.65			16.18±10.28***		
Hb	14.28±1.22	14.00±1.68	14.00±0.00	10.01±1.79	11.20±1.77	13.10±1.97
(g dL^{-1})	14.09±0.97			11.44±1.84***		

*Values are Mean±SE; ***P<.001 when compared to Control; Hb=Hemoglobin; BLL=Blood Lead Level.*

4. DISCUSSION

To evaluate adverse effects of lead exposure on occupationally exposed group, a selective cohort of individuals exposed to lead due to different work environment were selected for the study. The correlation between blood lead level (BLL) and Hb level was studied with respect to ALAD genotype polymorphism. In this study, we found significantly higher levels of lead in blood among workers as compared to control individuals. According to the US Center for Disease Control and Prevention (CDC), the BLL is considered high if it is more than 10 μg dL^{-1}. However, evidence indicating that some health effects can also occur below this threshold is accumulating [13]. Studies suggested that health effects may become apparent at concentrations of <5 μg dL^{-1} [14,15].

Lead inhibits ALAD, COPRO-O and FERRO-C in the heme synthesis pathway and effects on ALAD being most profound [16]. ALAD is a key enzyme in heme synthesis, and a genetic variant of ALAD (rs1800435 C/G) denoted as ALAD 1-2 or 2-2 allele was designated as a possible determinant of BLL and its toxicity [3,10,11,17]. The SNP is located in exon 4 and results in the exchange of Asparagine to Lysine at position 68 of the enzyme. The resulting change in amino acid sequence influences the charge of the isozyme ALAD 2-2 and may bind positively charged lead more tightly [10]. In corroborate with the present study, other researchers [3,10] also described that the ALAD 1-2 or 2-2 genotype is less common than ALAD 1-1 and has a heterogeneous distribution among different populations [3]. The allele frequency of both the ALAD 1-2 and ALAD 2-2 together in lead-exposed workers (n=200) was 20.16% in the present study.

The lower level of Hb was observed by various scientists [5,18-20] in rats and human with high BLL. It has been suggested that a genetic polymorphism in the human ALAD gene may cause certain individuals to be more sensitive to lead toxicity than others [3,21]. Moreover, Ray [5], recommended for consideration of hematological parameters which can be affected by gene polymorphism in lead toxicity study. It was observed that BLL was affected by ALAD genotype and can impact hematopoiesis which was resulted in the alteration of Hb content. It was also noted that ALAD 1-1 was having higher BLL than ALAD 1-2 or 2-2 polymorphic forms in this study. The similar findings were also observed in the studies of other scientists [2,22, 23].

In the present study, the significant correlation was also observed between ALAD genotype and Hb level in occupationally lead-exposed individuals. Hb level in ALAD 1-1 individuals, was found to be lower as compared to the ALAD 1-2 and 2-2 polymorphic forms. It may be due to the more amount of free lead present in the blood of workers having ALAD 1-1 genotype can destruct Hb production directly or abridged erythrocyte survival. It is hypothesized that workers with ALAD 1-1 genotype were more vulnerable to the harmful effects of lead exposure as compared to ALAD 2-2 where lead bound strongly to ALAD assembly and hence could not destruct Hb or RBC directly. This could be one of the causes of the higher concentration of Hb in workers having ALAD 2-2 genotype as compared to workers having ALAD 1-1 genotype in the present study. This predictive pattern indicated that toxic levels of lead in the blood might be related to early stages of anemia for ALAD 1-1 genotype. Various studies [5,13,20] reported the exposure of lead at very low concentrations decreases the Hb content in the blood which is in accordance to present study. So, these findings accomplished the association between occupational lead exposures and alteration in Hb level as well as the influence of genetic status of ALAD genotype in the development of adverse effects. The present study had given a clear evidence of BLL

with Hb and impact of ALAD gene polymorphism. However, some limitations are also inherent in this study. Due to a limited number of samples, the ALAD 2-2 genotype frequency was found inadequate in the studied population. We did not measure other possible gene-gene interactions likewise the roles of the Vitamin D receptor gene, Metallothionein 2A gene which is also important in lead toxicity study but the present study comprises the supporting outputs to prospective in-depth study related to lead toxicokinetic.

5. CONCLUSION

In the present study, the significant correlation was found between ALAD genotype and Hb level in occupationally lead-exposed individuals. Hb level in ALAD 1-1 individuals is low as compared to other two polymorphic forms. Elevated BLL but normal Hb content were found in the individual having ALAD 2-2 genotype due to efficiently lead binding effects of the ALAD 2-2 genotype with no associated hematotoxicity, though there was a long duration of exposure. The results also revealed that ALAD polymorphism might interfere with BLL and hence can alter the toxic biological effects of lead. It is well reported that ALAD 1-1 genotype leads to the low affinity of ALAD enzyme for lead cation. Thus, unbound lead cations circulate in the biological system and affect vital organs as well as the process like hemopoiesis which was evident in our study where individuals with ALAD 1-1 genotype showed low Hb level. So, to deal with health-related issues more efficiently in occupationally lead exposed workers, it is necessary to pay attention to genetic polymorphism of ALAD gene. Current observation on limited number of exposed individuals, apparently have emphasized on a larger study so as a regulatory draft for safety of occupationally exposed individuals based on their genotype can be suggested.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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