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International Journal of Clinical Biochemistry and Research

Journal homepage: https://www.ijcbr.in/

Original Research Article

Estimation of urinary delta aminolevulinic acid levels in gasoline and petrol pump workers as an index of lead exposure

Neelam Patil[1], Siddhesh Thorat[1], Sachin Patharkar[1,*], Alka Nerurkar[1], Umesh Shinde[1], Laxman Chougule[1], Jalinder Sanap[1], Kalpana Shinde[1]

[1] *Dept. of Biochemistry, T.N.M.C & B.Y.L Nair Ch. Hospital, Mumbai, Maharashtra, India*

ARTICLE INFO

Article history:
Received 28-10-2021
Accepted 22-12-2021
Available online 05-01-2022

Keywords:
Estimation of Urinary Delta Aminolevu
ALA- U- Urinary ALA
ALA- D- Amino Levulinic Acid-Dehydratase

ABSTRACT

Lead poisoning is a phenomenon which with growing globalization is being a subject of worry.ALA i.e Amino levulinic acid is a precursor of hemoglobin, which is synthesized in mitochondria by two main components succinyl Co-A and glycine in presence of ALA-S i.e. amino levulinic acid-synthase. Urinary ALA (ALA-U) has been a recommended biomarker for lead exposure. Inhibition of Amino levulinic acid-dehydratase (ALA-D) results into activation of ALA-S which further synthesizes ALA, excess of ALA is accumulated in the blood, plasma, urine. Present manuscript is focused on the estimation of levels of ALA in the urine of gasoline and pertol pump workers, by acidifying the urine to extract out ALA and reading it colorimetrically as they are exposed to fumes released by gasoline, petrol, and petroleum products which contains lead. Awareness and safety measures such as protective masks and gears should be provided by the respective organisations to the workers.

1. Introduction

Lead is one of the oldest known and most widely studied occupational and environmental toxins. Lead (Pb) is a widespread toxic metal in the human environment, getting there as a result of industrial processes (production of batteries, paints, varnishes, anti-detonation agent in gasoline).[1] Although USA and the European Countries have banned lead as an additive to consumer fuel for road-going vehicles, lead continues to be used in petrol in many countries including India. Petrol station workers are constantly in contact with leaded benzene and gasoline during their daily lives. Lead in the workplace comes from emissions in the form of fine particles that are inhaled and absorbed through the lungs, by ingestion, and through dermal exposure.

The ability of lead to pass through the blood-brain barrier is due in large part to its ability to substitute for calcium ions. Within the brain, lead-induced damage in the prefrontal cerebral cortex, hippocampus, and cerebellum can lead to a variety of neurological disorders, such as brain damage, mental retardation, behavioural problems, nerve damage, and possibly Alzheimer's disease, Parkinson's disease, and schizophrenia. At the molecular level, lead interferes with the regulatory action of calcium on cell functions and disrupts many intracellular biological activities. Experimental studies have also shown that lead exposure may have genotoxic effects, especially in the brain, bone marrow, liver, and lung cells.[2]

This article addresses the results of retrospective research carried out with attendants at gas station and those who are professionally exposed to inorganic lead.

* *Corresponding author.*
E-mail address: biochemist95@yahoo.com (S. Patharkar).

https://doi.org/10.18231/j.ijcbr.2021.058

2. Aim and Objectives

2.1. Aim

To estimate the urinary delta aminolevulinic acid levels in gasoline/petroleum workers as an index of lead exposure.

2.2. Objective

The main objectives of this project are -

1. To estimate the urinary delta aminolevulinic acid levels in gasoline/petrol pump workers.
2. To categorize the observed values into normal, acceptable, high, and dangerous levels on the basis of reference values and then compare it.
3. To educate the community of major health problems associated with elevated lead levels, the paths of lead exposure in their environment and ways they can protect themselves and their families from lead exposure.

2.3. Study design

A cross-sectional pilot observational study in six different petrol pump stations of Mumbai.

2.4. Setting

Study was designed and carried out in a Tertiary Care Hospital in Mumbai.

2.5. Sample size

80 petrol pump workers.

2.6. Duration of study

May 2018 to May 2020

Sample size was decided on the basis of formula:

$n = 4pq/l^2$

where l is permissible error in the estimation of new statistics, p is positive character, and q is 1 − p. Prevalence to estimate sample size found from previous studies of own institute, and National journals.

2.7. Sampling method

Urine samples of 80 petrol pump workers with due informed written consent were collected with their names, area, age, and daily working details, by random sampling with due ethical considerations. Samples were collected from different petrol pump workers in suburban of Mumbai. Urine samples were collected in 15-mL plastic containers, covered with brown paper, exercising standard precautions. First morning midstream urine samples were collected after local area cleaning. Each individual was interviewed using a standard questionnaire. Information regarding their working environment, personal protective equipment, personal hygiene and habits and working hours/day was collected. Their urine samples were analyzed for δ-ALA by Ehrlich method in which acidic urine reacts with n -butanol and δ-ALA is converted to its pyrrole at pH 6.8. The pyrrole reacts with Ehrlich's reagent to form red colour, which is extracted with chloroform and read colorimetrically.[3] The level of urinary δ-ALA was expressed as mg/L.

Comparing the method with other methods like ion exchange chromatography, the method discussed by Tomokuni et al., it is found that this method being colorimetric is easy, rapid, and accurate as all interfering substances are removed by butanol extraction.[4,5] The procedure was standardized, and graph was plotted prior to use on subjects.

3. Observation and Results

The study was done on 80 petrol pump workers.

According to the reference value i.e. (< 5mg/l), the result is divided in categories as higher and lower than reference values, higher is again sub-divided as Acceptable – high exposure level (5 or > 5 -20mg/l) and Dangerous -very high exposure level (20 or >20 -40mg/dl).

Out of 80 workers- 72 (90%) were categorized as above reference level and 8 (10%) were considered as below reference level.

Table 1: Below and above levels of δ ALA according to reference value

Statistical parameter	ALA below reference level	ALA above reference level
No. of sample	8	72
Percentage of exposure	10%	90%
Acceptable-high exposure level	-	69
Dangerous - very high exposure level	-	3

Out of the above calculated 72 workers (ALA above reference level)-

1. 69 (95.8%) were categorized as Acceptable -high exposure level and
2. 3 (4.20%) were categorised as Dangerous -very high exposure level.

4. Discussion

Delta amino levulinic acid is an essential precursor of heme. During biosynthesis δ -ALA goes through a series of transformations in the cytosol and finally gets converted to Protoporphyrin IX inside the mitochondria. This protoporphyrin molecule chelates with iron in presence of

Table 2: Acceptable (high exposure level) and Dangerous (very high exposure level).

Statistical parameter	Acceptable (high exposure level)	Dangerous (very high exposure level)
No. of sample (out of 72)	69	3
Percentage of exposure	95.8%	4.2%

enzyme ferrochelatase to produce Heme. The urinary δ -ALA levels increases due to lead poisoning.

This principle is used to detect lead exposure by measuring the urinary δ- ALA.[6,7] The quantitative estimation of δ-ALA is based on the principle of reactivity of pyrroles with p-dimethylaminobenzaldehyde. Though normal concentrations usually give only faint yellow or faint red colours elevated ALA concentrations were indicated by reddish colour in chloroform. Interfering substances in urine which react with Ehrlich reagent to form red colour and with the formation of pyrroles and aldehydes were removed by n-butanol extraction. Small amounts of Ehrlich positive substances which escaped the n-butanol extraction formed a red colour on addition of Ehrlich's reagent, but this never entered the chloroform phase. The specificity is good enough to use this method for screening for lead exposure.[8] The ALA pyrrole complex is formed with ethyl acetoacetate, which forms chromophore with Ehrlich reagent and extracted with chloroform.[9]

Increased urinary δ-ALA levels found in exposed subjects in the present study might be the impact of low-level long-term exposure of gasoline, petrol and petroleum products reinforces the notion that δ-ALA can serve as a surrogate marker for lead exposure.[10]

In 92.5% of exposed workers urinary δ-ALA levels obtained were above reference levels, it is a clear indicator of cumulative lead exposure and appears to be directly related to duration of employment at the work units, since almost 90% of them had 8 and above years of experience. Chronic lead exposure as a culprit for higher δ-ALA levels was also corroborated by the observation that levels vary with duration of employment. Urinary δ-ALA levels in workers who had served for many years were higher to those served for few years.

Lifestyle factors other than the occupational settings can influence the exposure of a toxicant. Such factors usually include smoking and alcohol consumption. In this study, the effect of alcohol on urinary δ-ALA levels of exposed subjects was considered and alcohol-consumption subjects displayed increased levels than their non-alcohol consuming peers and this is in line with other reports.[11] The role of alcohol in blood lead levels is still unclear and is still a subject of controversy. They argue that the equipment sometimes contains brass or gunmetal that has low but significant amounts of lead. Thus, it is possible to assume that the same argument might hold true for the observed increased urinary δ-ALA in alcohol-consuming exposed subjects.

After this study the results were explained to the participant's and necessary precautions were suggested which includes avoiding eating or chewing things like tobacco, regularly checking the blood lead levels, washing their hands and wearing a protective mask while at gas station so that the fumes released from the gasoline and petroleum products should not enter the respiratory tract, to stop rapping foods in newspapers, to clean their self's up before leaving specially hands as the more amount of gasoline is in contact with hands etc. By taking necessary precautions we can reduce the lead exposure to an extent as prevention is always better than cure.[12]

4.1. Other suggested precautions

1. Washing of clothes by gasoline and petrol pump workers immediately once home.
2. Wash yourselves thoroughly after the work hours, as petrol fumes could be still is present.
3. Mop floors often, and use damp clothes as dry mops spread dust to clean windowsills. Pour dirty water into the toilet.
4. Petrol pump workers should use protective mask while on duty.
5. Hand gloves should be made mandatory for petrol pump workers as there is direct contact of hands to petroleum products.

5. Conclusion

We conclude that the prevalence of lead exposure in gasoline and petrol pump workers in Mumbai is very high. Good hygienic practices and necessary precautions should be followed to avoid its ill effects on human body.

6. Future Scope

1. Measurement of blood ALA levels to compare with urinary ALA level as an index of lead exposure in gasoline and petrol pump workers.
2. Measurement of haemoglobin and correlation with urinary ALA level.
3. To measure the contribution of alcohol consumption in the increasing levels of lead exposure.

7. Source of Funding

Department Fund, Department of Biochemistry, T.N.M.C and B.Y.L Nair Charitable Hospital.

8. Conflict of Interest

Authors have declared that no conflict of interest exists.

9. Acknowledgement

We acknowledge Department of Biochemistry, TNMC and BYL Nair hospital for funding this project.

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Author biography

Neelam Patil, Associate Professor

Siddhesh Thorat, MSc Student

Sachin Patharkar, Associate Professor

Alka Nerurkar, Professor and Head

Umesh Shinde, Junior Scientific Officer

Laxman Chougule, Senior Laboratory Officer

Jalinder Sanap, Lab Technician

Kalpana Shinde, Junior Scientific Officer

Cite this article: Patil N, Thorat S, Patharkar S, Nerurkar A, Shinde U, Chougule L, Sanap J, Shinde K. Estimation of urinary delta aminolevulinic acid levels in gasoline and petrol pump workers as an index of lead exposure. *Int J Clin Biochem Res* 2021;8(4):274-277.