



Study of Estimation of Urinary Delta Aminolevulinic Acid Levels as an Indicator of Lead Exposure in Pottery Workers

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NJP and SAP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SRK and PNS managed the analyses of the study. Author URS managed the literature searches. Author AVN revised the final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Lead toxicity is a significant environmental hazard and has widespread ill effects on human body. In this study we detected the lead exposure by measuring urinary delta aminolevulinic acid levels. The activity of delta aminolevulinic acid dehydratase enzyme is inhibited significantly by lead, which leads to an increase in urinary δ -ALA excretion. Hence we can use estimation of urinary δ -ALA levels as a surrogate marker of lead exposure in pottery workers.

Aim: To estimate the urinary delta aminolevulinic acid levels in pottery workers as an index of lead exposure.

Materials and Methods: The study was done on 85 pottery workers of age group between 18 to 50 years with their brief history related to lead exposure. Their urine samples were analysed for δ -ALA by Ehrlich method.

Results: According to our observation, it was found that out of 85 urine samples; 58 samples showed increased urinary δ -ALA levels (68.23%).

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

Keywords: Urinary delta aminolevulinic acid (δ -ALA); blood lead levels (BLL); lead poisoning in pottery workers.

1. INTRODUCTION

Lead exposure accounts for about 0.6% of burden of disease in the world population [1]. The pottery area and furnace are both located in small areas in rundown shanties which are basically unhygienic and filthy. Pottery activities are carried on the floor and a wooden loft built overhead which is also a make shift place for members to sleep and eat. Painting, finishing and glazing the pottery with lead oxides is dangerous for these workers. Preparation of enamel and its application also poses risk for inhalation or ingestion of lead oxide. The baking process that sets the enamel releases significant amount of lead to the atmosphere. Also, the lead mixture is dangerous for skin of the potters as it is in direct contact with it [2]. The studies related to the lead exposure and its toxic effects are mainly done in the foreign countries considering their lifestyle, environment and surrounding conditions [3]. Very less work related to lead exposure and its parameters is done in India. Thus it is very significant to study lead exposure considering our lifestyle, environmental conditions. Lead exposure can be detected by estimating the blood lead levels but collection of blood samples, analysis of the blood sample is a quite tedious and time consuming procedure. It is known that heme synthesis pathway is affected by lead and production of haemoglobin is decreased leading to anaemia [4]. Heme synthesis pathway is affected by changes in the levels of necessary enzymes required for heme synthesis [5]. One of the effects of these changes is the increased levels of delta aminolevulinic acid in urine [6]. A simple colorimetric method can be used to estimate the delta aminolevulinic acid levels.

1.1 Aims and Objectives

The present study was conducted to find out the prevalence of lead poisoning among the pottery workers, in six different pottery workshops of Mumbai, by estimating the urinary δ -ALA levels as an index of lead exposure. Our study also aims to educate the pottery workers about major

health hazards associated with lead exposure and prevention of them by following proper health guidelines and necessary precautions.

2. MATERIALS AND METHODS

2.1 Study Design

A cross-sectional pilot observational study in six different pottery workshops of Mumbai.

2.2 Setting

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

Our study is Cross sectional observational study,

In order to estimate the required sample size, we need to know the following [6].

p: The prevalence of the condition/ health state. If the prevalence is 66.66% [7], it may be either used as such (66%), or in its decimal form (0.66).

q: i. When p is in percentage terms: (100-p)

ii. When p is in decimal terms: (1-p)

d (or l): The precision of the estimate. This could either be the relative precision, or the absolute precision / is permissible error in the estimation of new statistics We have taken it as 10%.

Z α [Z alpha]: The value of z from the probability tables. If the values are normally distributed, then 95% of the values will fall within 2 standard errors of the mean. The value of z corresponding to this is 1.96 (from the standard normal variate tables).

The formula for estimating sample size is given as:

$$N = \frac{(Z\alpha)^2 [p * q]}{l^2}$$

where the symbol ^ means 'to the power of'; * means 'multiplied by' that is, "Z-alpha squared

into pq; upon d-square” substituting the values of Za, we get:

$$N = \frac{(1.96)^2[p * q]}{d^2}$$

We can round off the value of Za (1.96) to 2, to obtain:

$$N = \frac{(2)^2[p * q]}{d^2}$$

or,

$$N = 4pq / d^2 \text{ that is, "4 pq by d-square"}$$

$$n = 4pq //$$

Sample Size: 85 pottery workers

Sampling Method: Random Sampling

Duration of Study: May 2017 to May 2019.

Samples were collected from six different pottery workshops of Mumbai. Urine samples were collected in 15-mL plastic containers, covered with brown paper with 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 [8].

Their urine samples were analyzed for δ-ALA by Ehrlich method. In this method 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. The level of urinary δ-ALA was expressed as mg/L. Comparing this method with other methods like ion exchange chromatography it was found that this method being colorimetric is easy, rapid, and accurate as all interfering substances are removed by butanol extraction [9]. The procedure was standardized, and graph was plotted prior to use on subjects.

3. RESULTS

According to the reference value, i.e. (<5 mg/L), the result was divided into two categories higher and lower than reference values. According to our observation, it was found that out of 85

workers; 58 (68.23%) workers were categorized as above reference level with mean ± SD value of 12.14 ± 3.19 and remaining 27 (31.76%) workers were considered as below reference level with mean ± SD value of 3.56 ± 1.87 (Table 1). The prevalence of lead exposure among our study population is 68.23%.

3.1 Statistical Analysis

The main outcome parameter urinary δ-ALA level is a continuous scaled data. To find out the prevalence of lead exposure we converted this data into categorical data depending upon the reference range of urinary δ-ALA levels. Hence only percentage of high exposed individuals was calculated. No other statistical test is required.

$$\text{Standard error of proportion (S.E.P)} = \sqrt{pq/n} = \sqrt{68 \times 32 / 85} = 5.05$$

So, 95% confidence interval 63.18 –73.28

4. DISCUSSION

Lead is considered to be highly toxic, exposure to lead salts even for a short period of time or presence of lead salts in the human body even in small amounts causes severe harmful effects. In this study we detected the lead exposure by measuring the urinary delta aminolevulinic acid levels. Lead exposure inhibits three enzyme activities in heme synthesis. The affected enzymes are aminolevulinic acid synthetase, delta-aminolevulinic acid dehydratase (ALAD) and ferrochelatase [10]. Urinary δ-ALA estimation is a surrogate biomarker of blood lead level. Urinary δ-ALA is normally excreted in small amounts in urine, and levels increase with increased lead exposure. As the activity of enzymes involved in heme synthesis pathway decreases, the concentration of urinary δ-ALA increases during lead exposure. By measuring the urinary δ-ALA we can detect the lead exposure [11]. The quantitative estimation of δ-ALA is based on the well-known reactivity of pyrroles with p-dimethylaminobenzaldehyde. Elevated ALA concentrations were indicated by a reddish colour in chloroform, while normal concentrations gave faint yellow or faint red colours. The interfering substances in urine which react with Ehrlich reagent to form red colour and also contains some substances which interferes with the formation of pyrroles and aldehydes are 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 ALA pyrrole complex is formed with ethyl acetoacetate, which forms chromophore with Ehrlich reagent and extracted with chloroform. 85 randomly collected samples were analysed by the procedure. It is known that random urine specimens from normal adult contain less than 5 mg ALA per litre urine. By considering this as reference value, the analysed samples were categorised into ALA below reference level and ALA above reference level, ALA concentration more than 5 mg/litre considered in ALA above reference level group and ALA concentration less than 5 mg/litre is considered in ALA below reference level group. Nearly 68.23% workers have shown elevated levels of ALA that means more than half of the population of workers is already being exposed to lead. Random urine specimens from normal

individuals contains around 0.2 mg δ -ALA per 100 mL urine. Comparing with other methods like HPLC, method derived in 1987, this method is very easy, rapid and accurate as all interfering substances are removed by butanol extraction [12].

The present study examined the association between U- δ -ALA levels of workers exposed to lead and duration of exposure in pottery workers. Increased urinary δ -ALA levels are obtained from about 68.23% of exposed workers, which is a clear indicator of cumulative lead exposure (Fig. 1 and Table 1).

It also appears to be directly related to the duration of employment at the work units, since 42 workers had more than 12 years of experience (Table 2).

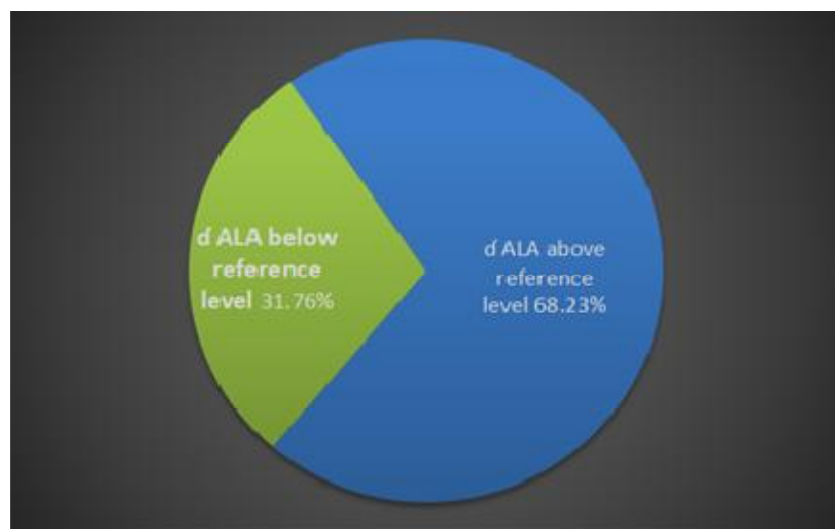


Fig. 1. Percentage of exposed workers with urinary δ -ALA levels

Table 1. Statistical parameters

Statistical parameter	ALA below reference level (<5 mg/L)	ALA above reference level (5 or >5–20 mg/L)
No. of samples (n=85)	27	58
Percentage of exposure	31.76%	68.23%
Mean	3.56	12.14
Standard deviation	± 1.87	± 3.19

Table 2. Duration of exposure/employment (for workers who fall in above reference category)

Duration of exposure/employment	Number of workers
>12 years and more	42
>6–12	9
<6 years	34

Table 3. Age distribution of workers

Age in years	Urinary δ -ALA levels <5 mg/L	Urinary δ -ALA levels >5 mg/L
15–20	3	2
21–30	11	19
31–40	6	21
41–50	4	14
51–55	3	2

In the present study predominantly the exposed subjects in age group 31–40 years followed by 21–30 years have shown increased urinary δ -ALA levels (Table 3).

The present study re-emphasizes the fact that chronic lead exposure is responsible for higher δ -ALA levels as the observation reveal that levels vary with duration of employment (Table 2).

Urinary δ -ALA levels in the workers who had served for many years were higher than in those served for few years and below. This finding is consistent with other reports that show urinary δ -ALA of especially of workers exposed to lead increases with an increase in the duration of exposure [13]. Evidence for the contribution of lead exposure to elevated urinary δ -ALA levels comes from the observation that 31.76% of less-to-non-exposed subjects (as per the details obtained from the workers) exhibited below reference level range, and none of them had high levels (Table 1 and Fig. 1). After this study the results were explained to the pottery workers and necessary precautions were suggested which includes employee information and training. Stringent personal hygiene, use of proper personal protection including protective clothing, awareness of hazardous materials, appropriate safety measures and hygiene facilities to be provided on site was also emphasized. The prevention programs that control or eliminate sources of exposure of lead poisoning are the only effective way to prevent the toxic effects associated with lead exposure [14].

5. FUTURE SCOPE OF THIS STUDY

- Measurement of blood ALA levels to compare with urinary ALA level as an index of lead exposure.
- Measurements of blood lead levels and correlate it with urinary ALA level.

6. CONCLUSION

The study is very significant as results obtained from the study show; 68.23% that is more than

half population of pottery workers are already being exposed. Necessary precautions can reduce the blood lead levels. Stringent personal hygiene, awareness of hazardous materials, and appropriate safety measures may reduce the health risks of repeated exposures. Also educating the people about the health effects, symptoms, and risk factors due to lead exposure is of prime importance.

CONSENT AND ETHICAL APPROVAL

Urine samples of 85 pottery workers with due informed written consent were collected with their brief history by random sampling with due ethical considerations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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