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Determination of delta aminolevulinic acid levels in urine; a sensitive indicator of lead exposure in construction workers

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Abstract: *Background:* Occupational exposure is a major source for lead poisoning in adults. Lead toxicity disrupts the functions of the digestive system, nervous system, respiratory system, reproductive system, etc. Lead decreases the activity of δ-aminolevulinic acid dehydratase which increases the excretion δ-aminolevulinic acid. The present study is based on this principle by which lead exposure is detected by measuring the urinary δ-ALA levels in construction workers *Aim:* To estimate the urinary delta aminolevulinic acid levels in construction workers as an index of lead exposure. *Materials and methods:* The study was done on 55construction workers of age group between 18 years and 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 the 55 urine samples; 16 samples (29.09%) had urinary δ-ALA levels below reference value (<5 mg/L), while 39 samples showed increased urinary δ-ALA levels (70.91%); out of these, 32 workers (82.05%) fell in the moderate-to-high exposure level category (5 or >5 to 20 mg/L), 7 workers (17.94%) fell in the dangerous - very high exposure level category (20 or >20 to 40 mg/L). *Conclusion:* We conclude that there is high prevalence of lead exposure in construction workers in Mumbai, and necessary precautions need to be taken to avoid it.

Keywords: Blood lead levels (BLL), Lead poisoning in construction workers, Urinary delta aminolevulinic acid (δ-ALA).

Introduction

Lead poisoning is one of the most common diseases of toxic environmental origin and accounts for about 0.6% of the global burden of disease. The Institute for Health Metrics and Evaluation (IHME) estimated that in 2017, lead exposure will be accounted for 1.06 million deaths and 24.4 million years of healthy life lost (disability-adjusted life years (DALYs)) worldwide due to long-term effects on health [1].

Lead exposure among construction workers is an issue of growing concern in occupational health [2]. Several episodes of severe lead poisoning among construction workers have been reported. Detection of lead exposure at early stage may be helpful to prevent from its toxicity. The studies related to the lead exposure, its effects and tolerance levels for lead are mainly done in the foreign countries considering their lifestyle, environment and surrounding conditions [3-5]. Very less work related on 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 which causes decreased production of haemoglobin and condition appears as anemia [6-7].

Heme synthesis pathway is affected by changes in the levels of necessary enzymes required for heme synthesis [8-9]. One of the effects of these changes is the increased levels of delta aminolevulinic acid in the urine [10].

It is also necessary to educate the people about the health effects, symptoms, and risk factors due to lead exposure as well as the required precautions [11].

Aims and Objectives: The present study was conducted to find out the prevalence of lead poisoning among the construction workers, in suburban areas of Mumbai, by estimating the urinary δ-ALA levels as an index of lead exposure and to educate the community about major health hazards associated with lead exposure and protection from it.

Material and Methods

Study Design: A cross-sectional pilot observational study in Mumbai suburban localities.

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

Sample Size: 55 construction workers. 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 $1 - p$. Permissible error or prevalence found from old thesis of own college, and national journals.

Sampling Method: Random Sampling

Urine samples of 55 construction 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 locations 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 color, which is extracted with chloroform and read colorimetrically. 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 [12]. The procedure was standardized, and graph was plotted prior to use on subjects.

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. Standard error of proportion (S.E.P) = $\sqrt{pq/n} = \sqrt{71 \times 29/55} = 6.11$

So, 95% confidence interval 64.79 – 77.01

Results

According to the reference value, i.e. (<5 mg/L), the result is divided into two categorieshigher and lower than reference values; which are again subdivided as high exposure level (5 or >5 to 20 mg/L) and very high exposure- dangerous level (20 or >20 to 40 mg/L). According to our observation, it was found that out of 55 workers, 39 (70.91%) workers were categorized as above reference level with mean ± SD value of 11.46 ± 4.29 and remaining - 16 (29.09%) were considered as below reference level with mean ± SD value of 3.92 ± 1.43 (Table 1). The prevalence of lead exposure among our study population is 70.91%.

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=55)	16	39
Percentage of exposure	29.09%	70.91%
Mean	3.92	11.46
Standard deviation	± 1.43	± 4.29

Out of the above-calculated 39 workers (ALA above reference level) - 32 (82.05%) were categorized as acceptable - high exposure level and 7 (17.94%) were categorized as very high exposure- dangerous level.

Discussion

Lead exposure is a serious problem having many detrimental health effects. In this study we detected the lead exposure by measuring one marker that is urinary delta aminolevulinic acid levels. The activity of delta aminolevulinic acid dehydratase is markedly decreased by lead, by which there is an increase in δ- ALA excretion in urine. By measuring the urinary δ- ALA we can detect the lead exposure. 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 usually gave only faint yellow or faint red colours. Urine usually contains many substances which react with Ehrlich reagent to form red colour and also contains some substances which interferes with the formation of pyrroles and aldehydes. These substances are removed by n-butanol extraction [13]. 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. The ALA pyrrole complex is formed with ethyl acetoacetate, which forms chromophore with Ehrlich reagent and extracted with chloroform.

55 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 [14]. By considering this as a reference value, the analysed samples were categorised into ALA below reference level and ALA above reference level, ALA concentration more than 5mg/litres considered in ALA above reference level group and ALA concentration less than 5mg/litre is considered in ALA below reference level group. Nearly 70.90% 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 contain in the order of 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 [15].

Lead exposure inhibits three enzyme activities in heme synthesis. The affected enzymes are aminolevulinic acid synthetase, delta-aminolevulinate dehydratase (ALAD) and ferrochelatase [16-17]. The estimation of urinary δ-ALA is considered to be a surrogate biomarker of blood lead level in construction workers. Urinary δ-ALA is normally excreted in small amounts in urine, and levels increase with increased lead exposure. The rise in the concentration of urinary δ-ALA during lead exposure is a primary function of the decreased activity of enzymes involved in the heme synthesis pathway.

The United States Occupational Health and Safety Administration (OSHA) medical surveillance guidelines provide information to physicians regarding the examination and evaluation of workers exposed to lead in four sections. Section one provides details on estimating BLLs, and its role in protecting workers from lead exposure. Section two outlines clinical manifestations of lead poisoning and the effects of lead intoxication on enzymatic pathways in heme synthesis. Section three outlines the recommended exposed worker medical evaluation. Section four provides detailed information on laboratory tests available for the monitoring of exposed workers [18].

The present study examined the association between U-δ-ALA levels of workers exposed to lead and duration of exposure in construction workers. Increased urinary δ-ALA levels are obtained from about 70.91% of exposed workers, which is a clear indicator of cumulative lead exposure (Table 1 and Fig.1). It also appears to be directly related to the duration of employment at the work units, since 36 workers had more than 12 years of experience (Table 2).

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

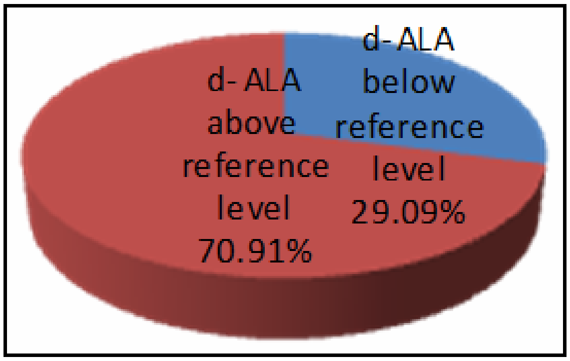


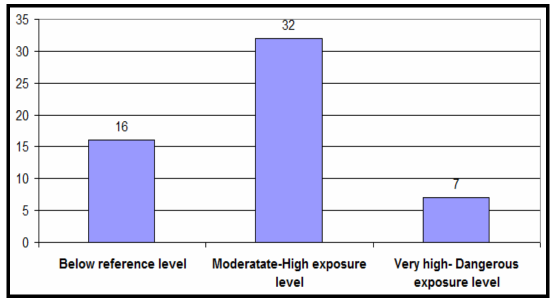
Table-2: Duration of exposure/employment (for workers who fall in above reference category)	
Duration of exposure/employment	**Number of workers**
>12 years and more	36
>6–12	6
<6 years	13

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	2	1
21–30	7	18
31–40	5	11
41–50	2	8
51–55	-	1

In the present study predominantly the exposed subjects in age group 21-30 years followed by 31-40 years have shown increased urinary δ-ALA levels (Table 3). The present study re-emphasises 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 2and Fig.2).

Urinary δ-ALA levels in the workers who had served for many years were higher than in those workers who served for few years and below. This finding is consistent with other reports that show urinary δ-ALA especially of lead workers increases with an increase in the duration of exposure [19-20].

Fig-2: Categorization of workers based urinary δ-ALA levels



It was observed that elevated levels of ALA were found in construction workers majorly because these are employed in commercial or residential renovation where lead-based paint may be disturbed. Abrasive blasting, welding, cutting and torch burning on surfaces coated with lead based paint can generate lead dust leading to inhalation and ingestion of lead. During the dismantling and transport of lead contaminated equipment and containment structures, as well as the clean-up of lead contaminated construction rubble and debris can re-entrain lead dust resulting significant lead exposure.

Evidence for the contribution of lead exposure to elevated urinary δ-ALA levels comes from the observation that 29.09% 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). This observation excludes the possibility that other factors might have contributed to the observed high levels of δ-ALA in exposed subjects.

After this study the results were explained to the construction workers and necessary precautions were suggested which includes employee information and training, Use of proper personal protection including protective clothing, shoe cover and gloves, avoiding the use of lead based paints, hygiene facilities to be provided on construction site.Primary prevention strategies that control or eliminate sources of exposure of lead remains the preeminent public health approach to address lead poisoning and the only effective way to prevent the abnormalities associated with lead exposure [21].

Future Scope of this study:

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

Conclusion

From the results obtained, it was concluded that, urinary delta aminolevulinic acid is a marker for detecting the lead exposure. The study sample represents the lower middle or middle socio economic strata. The study is very significant because such striking results were obtained from the study; 70.91%that is more than half population of construction workers are already being exposed. Hence it is concluded that we cannot stop lead exposure but we can prevent it. Necessary precautions can reduce the blood lead levels.

The symptoms for lead exposure may be absent in these construction workers but if the exposure persists for longer time, then it may affect their mental, social and physiological health and this can be prevented by following proper health guidelines and by necessary precautions.

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References

1. Lead poisoning and health [Internet]. *Who.Int.* 2020 [cited 12 April 2020]. Available from: *https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health*
2. Wani AL, Ara A, Usmani JA. Lead Toxicology: a review. *Interdiscip Toxicol,* 2015; 8(2):55-64.
3. CDC - NIOSH Update - Children of Construction Workers at Increased Risk for Lead Poisoning [Internet]. *Cdc.gov.* 2020 [cited 12 April 2020]. Available from: *https://www.cdc.gov/niosh/updates/leadpois.html*
4. Reynolds SJ, Fuortes LJ, Garrels RL, Whitten P, Sprince NL. Lead poisoning among construction workers renovating a previously deleaded bridge. *Am. J. Ind. Med.* 1997; 31:319-323.
5. Stephen M. Levin, Mark Goldberg. Clinical evaluation and management of lead-exposed construction workers. *Am. J. Ind. Med.* 2000; 37:23-43.
6. Andrade V, Mateus ML, Batoreu MC, Aschner M, dos Santos AP. Urinary delta-ALA: a potential biomarker of exposure and neurotoxic effect in rats cotreated with a mixture of lead, arsenic and manganese. *Neurotoxicol.* 2013; 38:33-41.
7. Kang HG, Bischoff K, Ebel JG, Cha SH, McCardle J, Choi CU. Comparison of blood lead and blood and plasma α-aminolevulinic acid concentrations as biomarkers for lead poisoning in cattle. *J Vet Diagn Invest.* 2010; 22(6):903-907.
8. Ahmed K, Ayana G, Engidawork E. Lead exposure study among workers in lead acid battery repair units of transport service enterprise. *J Occup Med Toxicol.* 2008; 3(30):1-8.
9. Mazumdar I, Goswami K, Ali S. Status of serum calcium, vitamin D and parathyroid hormone and hematological indices among lead exposed jewelry workers in Dhaka, Bangladesh. *Indian J ClinBiochem.* 2017; 32(1):110-116.
10. Sakai T. Biomarkers of lead exposure. *Ind Health.* 2000; 38(2):127-142.
11. Barman K. Assessment of Lead Exposure and Urinary-α-aminolevulinic Acid Levels in Male Lead Acid Battery Workers in Tamil Nadu. *J Health Pollut*, 2018; 8:6-13.
12. Tomokuni K, Ogata M. Simple method for determination of urinary delta aminolevulinic acid as an index of lead exposure. *Clin Chem* 1972; 18(12):1534-1536.
13. Wada O, Toyokawa K, Urata G, Yano Y, Nakao K. A simple method for the quantitative analysis of urinary delta-aminolevulinic acid to evaluate lead absorption. *Br J Ind Med,* 1969l; 26(3):240-243.
14. Tomokuni K, Ogata M. Normal levels of delta aminolevulinic acid in the spot urines of adult men, as measured by a simplified method. *Sangyo Igaku.* 1980; 22(2):92-95.
15. Ogata M, Taguchi T. High performance liquid chromatographic procedure for quantitative determination of urinary delta-aminolevulinic acid as indices of lead exposure. *International Archives of Occupational and Environmental Health.* 1987; 59(4):385-391.
16. Lubran MM. Lead toxicity and heme biosynthesis. *Ann Clin Lab Sci.* 1980; 10(5): 402-413.
17. Moore M, Goldberg A, Yeung-Laiwah A. Lead Effects on the Heme Biosynthetic Pathway Relationship to Toxicity. *Annals of the New York Academy of Sciences.* 1987; 514(1 Mechanisms of):191-203.
18. Lead: medical surveillance guidelines [Internet] In: Occupational Safety and Health Standards: 29 CFR1910. *Washington, DC: U.S. Department of*

Labor: Occupational Safety and Health Administration. [about 10 screens]. Available from: *https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10033*
19. Patharkar S, Benwal S, Nerurkar A, Patil N. Estimation of Urinary Delta Aminolevulinic Acid Levels in Garage Workers as an Index of Lead Exposure. *Indian journal of Medical Biochemistry,* 2019; 23(3):312-315.
20. Patharkar S, Chavan S, Phadke M, Patil N, Bokankar D. Estimation of Urinary δ Aminolevulinic Acid Levels (δ-ALA) In Children of Age Group 1 to 5 Years As An Index of Lead Exposure. *MRIMS Journal of Health Sciences,* 2018; 6(1):16-19.
21. Sanborn MD, Alan Abelsohn, Monica Campbell, Erica Weir.Identifying and managing adverse environmental health effects: 3. Lead exposure. *CMAJ,* 2002; (166):1287-1292.

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