International Journal of Biochemistry Research & Review

29(4): 12-16, 2020; Article no.IJBCRR.56742
ISSN: 2231-086X, NLM ID: 101654445

Estimation of Urinary Delta Aminolevulinic Acid (δ-ALA) Levels in Students of Age Group 15 to 25 Years as an Index of Lead Exposure

Sachin A. Patharkar[1], Suraj H. Jain[1], Alka V. Nerurkar[1], Neelam J. Patil[1*] and Pradeep N. Surve[1]

[1]Department of Biochemistry, Topiwala National Medical College and BYL Nair Charitable Hospital, Mumbai, Maharashtra, India.

Authors' contributions

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

Article Information

DOI: 10.9734/IJBCRR/2020/v29i430181
Editor(s):
(1) Dr. Chunying Li, Georgia State University, USA.
Reviewers:
(1) Edmond Puca, University Hospital Center, Albania.
(2) Ana Afonso, Universidade Nova de Lisboa, Portugal.
Complete Peer review History: http://www.sdiarticle4.com/review-history/56742

Original Research Article

Received 25 February 2020
Accepted 01 May 2020
Published 07 May 2020

ABSTRACT

Background: Lead poisoning is a serious and sometimes fatal condition. It occurs when lead builds up in the body. Students exposed to lead by eating junk foods wrapped in newspaper, the licking of fingers for turning the photocopies, book pages, printout etc. and use of same fingers for eating purpose. Additionally young population get exposed to lead by use of Kohl or Kajal eyeliners, some traditional ethnic medicines, time spent at firing ranges and some hobbies like wall paintings or jobs related to plumbing and soldering. Damage caused by lead poisoning cannot be reversed. Lead inhibits necessary enzymes required for heme synthesis, and this result in increased levels of delta-aminolevulinic acid (δ-ALA) excretion through urine.
Aim: To Estimate the urinary δ-ALA levels in the students of age group 15 to 25 years as an index of lead exposure.
Study Design: This is a cross-sectional study in Mumbai from a suburban locality.

**Corresponding author: E-mail: neelamb99@gmail.com;*

Methodology: The study was done on 70 students of age group between 15 years and 25 years with their brief history related to lead exposure. Their urine samples were analyzed for δ-ALA by using Ehrlich reagent.
Results: According to our observation, out of 70 students 46 student's (65.71%)urine samples shown δ-ALA level above reference level and remaining 24 (34.28%) were considered as below reference level.
Conclusion: The prevalence of lead exposure among our study population is 65.71%.
Clinical Significance: We conclude that there is high prevalence of lead exposure in students of age group 15 to 25 years in Mumbai and necessary precautions need to be taken to avoid detrimental effects of lead poisoning.

Keywords: Blood lead levels; lead exposure in students in Mumbai; Urinary δ ALA.

1. INTRODUCTION

Lead is a highly toxic metal and a very strong poison. Lead poisoning is a serious and sometimes fatal condition. It occurs when lead builds up in the body. Toxic exposure affects the brain and other vital organs, causing neurological and behavioral changes, gastrointestinal illness, and kidney impairment and interferes with heme synthesis. Lead is found in lead-based paints, including paint on the walls of old houses and toys. It is also found in art supplies, contaminated dust and ink used in printer, xerox machine ink. Lead poisoning usually occurs over a period of months or years. It can cause severe mental and physical impairment and can cause educational growth impairment in students [1,2,3].

Students exposed to lead by eating junk foods wrapped in newspaper, the licking of fingers for turning the photocopies, book pages, printout etc and use of same fingers for eating purpose. Additionally young population get exposed to lead by use of Kohl or Kajal eyeliners, some traditional ethnic medicines, time spent at firing ranges and some hobbies like wall paintings or jobs related to plumbing and soldering. Damage caused by lead poisoning cannot be reversed. Lead inhibits necessary enzymes required for heme synthesis, resulting in abnormal concentrations of heme precursors in blood and urine including delta-aminolevulinic acid (δ-ALA). Essentially, lead interferes with the activity of three enzymes: it indirectly stimulates the mitochondrial enzyme aminolevulinic acid synthetase; it directly inhibits the activity of the cytoplasmic enzyme aminolevulinic acid dehydratase; and it interferes with the normal functioning of intramitochondrial ferrochelatase. This result in increased levels of delta-aminolevulinic acid (δ-ALA) excretion through urine. Hence we planned to estimate urinary δ-ALA as a marker of lead exposure in study population [4,5].

1.1 Aims and Objectives

The present study was conducted to find out the prevalence of lead exposure among the students of age group 15 to 25 years, in a suburban Mumbai, by estimating the urinary δ-ALA levels as an index of lead exposure and to educate the the students about health hazards of lead exposure and protection from it.

2. MATERIALS AND METHODS

This is a cross-sectional pilot observational study in suburban Mumbai. Urine samples of 70 students with due informed written consent were collected with their names, age, gender and address details, by random sampling with due ethical considerations. Urine samples were collected in 15-mL plastic containers, covered with brown paper, exercising standard precautions. First morning midstream urine samples were collected after cleaning local area. Sample size was decided on the basis of formula: n = 4pq/l2 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.

Urine samples were analyzed for δ-ALA by using Ehrlich's reagent in which acidic urine reacts with n-butanol and δ-ALA was converted to its pyrrole at pH 6.8. The pyrrole reacted with Ehrlich's reagent to form red color, which was extracted with chloroform and read colorimetrically [6]. Comparing this 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 [7,8]. The procedure is standardized, and graph is plotted prior to use on subjects.

3. RESULTS

In present study, 70 randomly collected urine samples from predominantly male students of age groups 15 to 25 years were analyzed for δ-ALA levels. The analyzed samples were categorized into δ-ALA levels below reference value and δ-ALA levels above reference value. δ-ALA concentration equal to or more than 5mg/litre is considered as above reference value and δ-ALA concentration less than 5 mg/litre is considered as below reference value [6,7].

Out of 70 students 46 (65.71%) were categorized as having δ-ALA levels above reference and remaining 24 (34.28%) were considered as δ -ALA levels below reference values.

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. So calculated only percentage of high exposed individuals. No other statistical test is required.

Standard error of proportion S.E.P.

$$= \sqrt{\frac{pq}{n}} = \sqrt{\frac{66 \times 34}{70}} = 5.66$$

So, 95% confidence interval 60.05–71.37

4. DISCUSSION

In this study, we estimated the lead exposure in study group by measuring urinary marker, i.e., δ-ALA levels. The activity of δ-ALA dehydratase is markedly decreased by lead, by which there is an increase in δ-ALA excretion [9,10]. By measuring the urinary δ-ALA, we can estimate the lead exposure [11]. The quantitative estimation of δ-ALA is basically based on the well-known reactivity of pyrroles with p-dimethylaminobenzaldehyde. Elevated δ-ALA concentrations were indicated by a reddish color in chloroform, while normal concentrations usually gave only faint yellow or faint red color. Urine usually contains many substances, which react with Ehrlich's reagent to form red color, and it also contains some substances that interfere with the formation of pyrroles and aldehydes. These substances are removed by n-butanol extraction. Small amounts of Ehrlich-positive substances, which escaped the n-butanol extraction, formed a red color with the addition of Ehrlich's reagent, but this never entered into the chloroform phase. The only substance that behaved like δ-ALA was aminoacetone. Urinary aminoacetone levels in lead poisoning have been reported to be entirely normal. The specificity is good enough to use this method for screening lead exposure [12,13]. Normal urinary δ-ALA excretion by adult healthy men averaged 1.6 mg/l [2].

The highest level of urinary δ-ALA found in this study was 21.33 mg/l. This clearly indicate that the individuals are already at a high risk of lead exposure. Further 24 samples in this study have δ-ALA below reference level. This indicate we can prevent these individuals from detrimental effects of lead exposure by explaining necessary precautions.

It was clearly observed that elevated levels of δ-ALA found in urine of the students who had habits of eating noodles, canned foods and drinks, junk food wrapped in newspaper, by uses of xerox / printouts, licking the fingers with xerox / printout, habits of smoking and consuming alcohol.

After this study the results were explained to the participants and necessary precautions were suggested. These includes avoiding the use of canned foodstuffs and eating food wrapped in newspaper, minimizing the uses of xerox/ printout, avoiding the habit of licking the fingers to turn xerox/ printout pages, regularly checking the blood lead levels, washing their hands, drinking clean and filter water, avoid smoking or consuming alcohol. We can reduce the lead exposure to an extent bytaking necessary precautions.

Table 1. Statistical parameters

Statistical parameter	Urinary δ-ALA below reference level (≤5 mg/l	Urinary δ-ALA above reference level (>5 mg/l
*No. of samples	24	46
Percentage of exposure	34.28%	65.71%
Mean	2.88 mg/l	8.48 mg/l
Standard deviation)	± 0.9377	± 3.359

**urine from study population (students)*

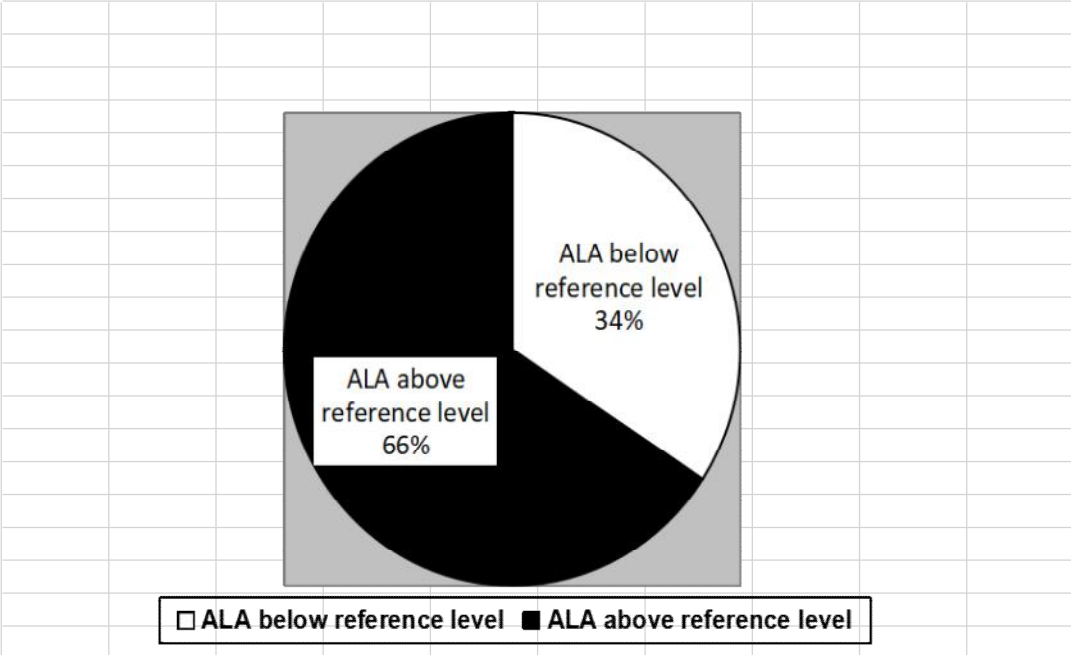

Fig. 1. Percentage of exposure according to urinary δ-ALA reference value

Table 2. Age distribution

Age in years	Urinary δ-ALA below reference level (≤5 mg/l	Urinary δ-ALA above reference level (>5 mg/l
15-20	6	26
21-25	18	20

Table 3. Habits of study population

Habits	Percentage
Eating the junk food wrapped in newspaper	95%
Licking behaviour	90%
Alcohol or smoking	10%

4.1 Future Scope

Measurements of blood lead levels and correlate it with urinary δ-ALA level and compare levels between male and female genders.

These kinds of studies will help us to establish new reference levels by considering changes in the environment, modification and removal of lead from paints gasoline etc.

5. CONCLUSION

The study sample represents young students, who are future of country. It is important to make them aware about lead exposure and it's detrimental effects in their daily routine. The results obtained from the study are 65.71% that is more than half of the study population of students have already been exposed to lead. It is now our duty to save the rest of the students from ill effects of lead exposure and therefore, it is important to educate them regarding standard precautions to avoid lead exposure.

CONSENT

All authors declare that written informed consent was obtained from the patient.

ETHICAL APPROVAL

All authors hereby declared that all experiments have been examined and been performed in accordance with the ethical standards.

ACKNOWLEDGEMENTS

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gould E. Childhood lead poisoning: Conservative estimates of the social and economic benefits of lead hazard control. Environ. Health Perspect. 2009;117(7): 1162–1167.
2. Chandramouli K, Steer CD, Ellis M, Emond AM. Effects of early childhood lead exposure on academic performance and behaviour of school age children. Arch. Dis. Child. 2009;94(11):844–848.
3. Advisory Committee on Childhood Lead Poisoning Prevention. Low Level Lead Exposure Harms Children: A Renewed Call for Primary Prevention. 2012:1–68. Available:http://www.cdc.gov/nceh/lead/ACCLPP/Final_Document_030712.pdf. [Accessed March 6, 2012]
4. Patharkar SA, Benwal SJ, Nerurkar AV, et al. Estimation of urinary delta aminolevulinic acid levels in garage workers as an index of lead exposure. Indian J Med Biochem. 2019;23(3):312–315.
5. Kappas A, Sassa S, Galbraith RA, Nordmann Y. In: Scriver CR,Beaudet AL, Sly WS, Valle D, editors. The metabolic basis ofinherited diseases. 7th Ed. USA: McGraw Hill. 1995;2103–59.
6. 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.
7. Katsumaro Tomokuni, Masayoshi Ichiba and Yukio Hirai. Measurement of urinary δ-aminolevulinic acid (ALA) by fluorometric HPLC and colorimetric methods. Industrial Health. 1992;30:119-128.
8. Cramer K, Selander S. Studies in lead poisoning: Comparison between different laboratory tests. Br J Ind Med. 1965; 22(4):311–314. DOI:10.1136/oem.22.4.311
9. Tanabe Y. Metabolism of delta-aminolevulinic acid (δ-ALA) and porphobilinogen in lead poisoning. I. Amounts of δ-ALA and PBP in the urine and blood. Jap. J. Nat.Health. 1959;28: 386-397.
10. Masana OGATA, Toyohiro TAGUCHI. Quantitative determination of urinary delta-aminolevulinic acid as an index of lead exposure by high performance Liquid Chromatography. Industrial Health. 1986; 24:259-264.
11. Patharkar S, Chavan S, Phadke MS, Patil N, Bokankar DK. 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 J Health Sciences. 2018;6(1):16-19.
12. Wada O, Toyokawa K, Urata G, Yano Y, Nakano K. A simple method for the quantitative analysis of urinary delta-aminolevulinic acid to evaluate lead absorption, Br. J. Ind. Med. 1969;26:240.
13. Katsumaro Toniokuni, Yuklo Hiral. Factors affecting determination of δ-aminolevulinate by use of ehrlich's reagent. Clin. Chem. 1986;32/1:192-193.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/56742