Original Article

Estimation Of Urinary δ Aminolevulinic Acid Levels (δ-ALA) In Children of Age Group 1 to 5 Years As An Index of Lead Exposure

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Abstract:

Background: Lead poisoning is very much common in India and has significant negative impact on general health of children. Lead inhibits necessary enzymes required for heme synthesis, this results in increased levels of delta aminolevulinic acid excretion through urine. O**bjective:** To find out the prevalence of lead poisoning among the age group 1 to 5 years, in a suburban Mumbai, Maharashtra, India.
Methods: This is cross-sectional pilot study in Mumbai suburban localities. The study was done on 60 randomly chosen children of age group 1 to 5 years with their brief history related with lead exposure. Their urine samples were analyzed for δ ALA by Ehrlich method.
Results: According to our observation it was found that out of 60 urine samples 20 samples have urinary δ-ALA levels below 0.2 mg/dl, while 40 samples showed increased urinary δ-ALA levels. Prevalence of lead exposure among our study population is 66.66% hence lead exposure is a serious public health issue.
Conclusion: we conclude that there is high prevalence of lead exposure in Mumbai and necessary precautions to be taken to avoid it.
Key words: Urinary delta (δ) ALA; Blood lead levels; lead poisoning in children**,** lead.

Introduction:

Lead exposure and poisoning has created more social concern and panic due to recent ban on leading noodles food brand in India. Lead poisoning is one of the most common and best-recognized childhood diseases of toxic environmental origin. Children around the world today are at risk of exposure to lead from multiple sources. Lead poisoning accounts for about 0.6% of the global burden of disease [1]. Numerous international conferences and declarations have recognized the importance of childhood lead poisoning and the need to intervene to prevent it.
It affects human beings as our normal food, water are contaminated with lead. Paint used for toys contain lead. Newspaper ink contains lead and our habit of wrapping food in newspapers generates potential source of exposure. Lead exposure, even at low levels, has a significant negative impact on general health, overall growth and development and educational outcomes. Lead may cause irreversible neurological damage as well as renal disease, cardiovascular effects, and reproductive toxicity. Blood lead levels once considered safe are now considered hazardous, with no known threshold [2].
More than 50 % children dwelling in Indian urban slums have blood lead levels above 10 µg/dl, 14% of them have seriously elevated blood lead levels above 20µg/dl [3].
Lead is heavy metal used by mankind for over 9,000 years. Lead occurs naturally in the earth's crust and is dispersed in small amounts into the environment by natural processes. It is also brought into the environment through human activities. Lead accumulates in the environment, is non-biodegradable and does not lose its toxicity over time [4]. Lead enters our environment through mining, smelting and carried by air deposits in soil and water, thus enters in food chain and drinking water, paint and toys; dust from plumbing material contains high lead levels [5].
Present study was conducted to find out the prevalence of lead poisoning among the age group 1 to 5 years, in a suburban Mumbai, Maharashtra, India by estimating the urinary δ ALA levels as an index of lead exposure and to educate the community about health hazards of lead exposure and protection from it.

Material and Methods:

This is cross-sectional pilot observational study in Mumbai suburban localities. The city of Mumbai divided into different zones and subzones, by random sampling. Study population is taken from Ville Parle (East). The study was done on 60 randomly chosen children both males and females of age group 1 to 5 years with their brief history related with lead exposure with due informed consent taken from parents..
This study is designed and carried out in the Department of Biochemistry, Topiwala National Medical College and

B.Y.L. Nair Charitable Hospital, Mumbai 400008, during the period 2015 May -2016 May. Sample size was decided on the basis of following formula:

$$n = \frac{4pq}{l^2}$$

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.

Urine samples were collected from children with exercising standard precautions. First morning midstream urine samples collected after local area cleaning. Their urine samples were analyzed for δ ALA by Ehrlich method in which acidic urine reacts with n-butanol, δ ALA was converted to its pyrrole at pH 6.8. The pyrrole reacted with Ehrlich's reagent to form red colour, which was extracted with chloroform and read colorimetrically[6].

Comparing the method with other methods like ion exchange chromatography, method discussed by Katsumrao 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.

Results:

According to our observation it was found that out of 60 urine samples 20 samples have urinary δ-ALA levels below 0.2 mg/dl, while 40 samples showed increased urinary δ-ALA levels. Prevalence of lead exposure among our study population is 66.66%. Four children included in this study were suffering from anemia having elevated urinary delta ALA concentration. In our study, among forty samples that showed increased levels of urinary δ-ALA levels, 19 were female children and 21 were male children, so apparently there was no sex difference, in lead exposure. 17.5% children had the habit of eating junk food like noodles, 17.5 % children had the habit of eating paint and nearly 32.5% children had the habit of hand-to-mouth behavior and repeated eating of non-food items. They all showed increased urinary ALA levels and thus considered as lead exposed children. Our study well co-ordinate with a major screening program called "Project Lead Free" was carried out by The George Foundation (TGF) during 1997-1999 to determine the blood lead levels among the population in seven major Indian cities (Bangalore, Calcutta, Chennai, Vellore, Hyderabad, Delhi and Mumbai). This study of over 15,000 children and 5,000 adults clearly indicated an environmental health crisis; it concluded that over 50% of the children below the age of 12 years living in urban environments have unacceptable blood lead levels of 10ug/dl or more. Further 14% of the children in these cities have seriously elevated levels of lead of 20ug/dl or more. The results of Project Lead-Free brought about greater awareness of the problem among the public, and more importantly, among policy makers in government[9, 10].

Table 1: Prevalence of Lead exposure among our study subjects

Urinary δ-ALA levels	Urinary δ-ALA levels below 0.2 mg/dl	Urinary δ-ALA levels more than 0.2 mg/dl
Number of study subjects	20	40
Percentage	33.34	66.66%
Mean	1.0605	4.9277
Standard deviation	0.7325	2.1142

Table 2: Sex Distribution of Lead exposure among Male and female children

Sex distribution	Male	Female
Number of study subjects	21	19
Percentage	52.5%	47.5%

From table 2, one can infer that there is no significant difference among males and females.

Table 3: Age Distribution

	Urinary δ-ALA levels below 0.2 mg/dl	Urinary δ-ALA levels more than 0.2 mg/dl
1 year	02	03
2 year	05	09
3 year	10	10
4 year	01	07
5 year	02	11

From the table 3, one can infer that lead exposure is comparatively high in all ages as comparared to their counterparts having Urinary δ-ALA levels below 0.2 mg/dl. And this proportion is specially increased in 4^{th} and 5^{th} year of age.

Table 4: Habits of study population

Habits	Percentage
Eating junk food like noodles	17.5%
Eating paint	17.5%
Hand-to-mouth behavior (putting toys in mouth)	32.5%

From table 4, one can infer that Hand-to-mouth behavior (putting toys in mouth) is leading cause of lead exposure.

Statistical analysis

If data type is categorical /qualitative/ enumeration, we calculate number and then percentage so therefore accordingly for this study number and percentage is calculated hence no other statistical test or software required.

Standard error of proportion $S.E.P = \frac{\sqrt{pq}}{n}$

$= \frac{\sqrt{66*34}}{60}$

= 6.11

So 95% confidence interval 54.44 to 78.88

Discussion:

In this study we detected the lead exposure by measuring urinary marker that is delta aminolevulinic acid levels (δ-ALA). The activity of delta aminolevulinic acid dehydratase is markedly decreased by lead, by which there is an increase in δ- ALA excretion [10, 11]. By measuring the urinary δ- ALA we can detect the lead exposure [12, 13]. The quantitative estimation of δ- ALA is basically 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 colour. Urine usually contains many substances which react with Ehrlich reagent to form red colour and also contains some substances which 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 colour on addition of Ehrlich's reagent, but this never entered the chloroform phase. The only substance which behaved like 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 for lead exposure.

Random urine specimens from normal individuals contain in the order of 0.2 mg δ - ALA per100 ml urine. It was clearly observed that the elevated levels of urinary δ- ALA were found in children who had the habits of eating paint, chalk, soil, junk food like noodles, and hand-to-mouth behavior i.e. repeated eating of non-food items. Children who live near the ongoing building construction, showed elevated urinary δ-ALA levels. 9 such samples were analyzed. This elevated urinary δ- ALA is because of the absorption of lead from dust, air and soil. After determining all this urinary δ-ALA levels the results were explained to the participant's parents and necessary precautions were suggested which include avoiding the use of lead based paints, regularly checking the blood lead levels.

Suggested Precautions [15]

- Regular cleaning and washing away of lead dust.
- Regular hand washing of children before eating.
- Avoid putting toys in mouth.
- Use wet clothes to mop floors, windows.
- Regular repair of peeling paint and always use lead free paints.
- Special precautions of pregnant women and children during painting and home repairs.
- Avoid use of products such as traditional medicines, herbs, spices cosmetics that could have lead.
- Always use tap water after 1 minute run, so it will help to clear lead from old plumbing, use cold tap water for drinking and cooking.

We cannot stop the lead exposure but we can prevent ourselves by certain precautions.

Conclusion:

We would like to conclude that in Mumbai suburban areas there is very high prevalence of lead exposure, in our pilot study it is nearly 66.66% and to reduce its exposure and prevent complications, it is necessary to educate the people about the health effects, symptoms, and risk factors as well required precautions.

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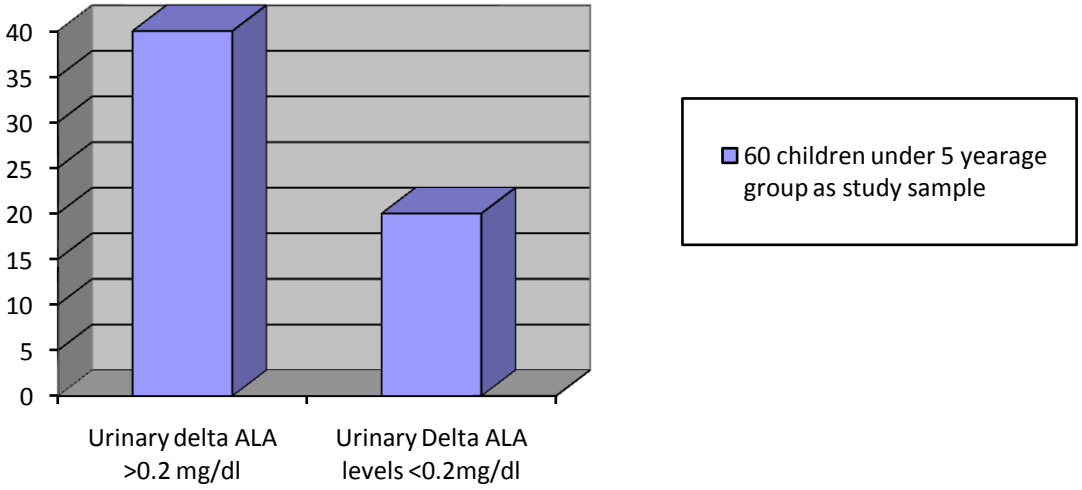

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