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Estimation of Lead Level in Blood among South Delhi Population: A Cross Sectional Autopsy Based Study

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Abstract

The widespread use of lead causes accumulation results in poisoning known as plumbism. The acceptable reference range for lead in human beings is 25μg/dl. Present study shows the blood lead levels of South Delhi population. 5 ml of blood was taken and digested followed by analysis using trace metal analyser (TMA). The results obtained were further categorized onthe basis of sex and age. Out of 250 autopsies 77 were females having mean blood lead level 0.41 μg/dl while rest 173 weremales having mean blood lead level 16 μg/dl. According to age-wise distribution the highest mean blood lead level is found in the age group of 41-60 years i.e. 20.73 μg/dl.

Keywords: Lead; TMA; Blood; Microwave Digestor; Poisoning.

Introduction

The widespread use of lead because of its properties leads to its poisoning termed as plumbism, colicapictonum, aturnism, devoncolic, or painters colic. It is a medical condition caused by increased lead levels in the body [1].

Symptoms include headache abdominal pain memory loss kidney failure, male reproductive problems, and weakness, pain, or tingling in the extremities. The symptoms in children's are different which includes loss of appetite, abdominal pain vomiting weight loss constipation anaemia kidney failure irritability lethargy learning disabilities and behaviour problems [2]. Children may also experience hearing loss, delayed growth, drowsiness, clumsiness or loss of new abilities specially speech skills [3].Symptoms in childrens may appear at lower blood lead levels [4].

One of the main causes for the pathology of lead is that it interferes with the activity of an essential enzyme called delta-aminolevulinic acid dehydratase or ALAD, which is important in the biosynthesis of heme, the cofactor found in hemoglobin [5]. Lead also inhibits the enzyme ferrochelatase, another enzyme involved in the formation of heme. Ferrochelatasecatalyzes the joining of protoporphyrin and Fe^{2+} to form heme [6]. Lead's interference with heme synthesis results in production of zinc protoporphyrin and the development of anemia [4]. Another effect of lead's interference with heme synthesis is the buildup of heme precursors, such as aminolevulinic acid, which may be directly or indirectly harmful to neurons. Lead interferes with the release of neurotransmitters, chemicals used by neurons to send signals to other cells [7]. It interferes with the release of glutamate, a neurotransmitter important in many functions including learning, by blocking N- methyl D -aspartic

acid (NMDA) receptors. The targeting of NMDA receptors is thought to be one of the main causes for lead's toxicity to neurons [8]. The current reference range for acceptable blood lead concentrations in healthy persons without excessive exposure to environmental sources of lead is less than 5 µg/dl for children and less than 25 µg/dl for adults [9].

Material & Methods used

In this study all deaths from south Delhi reported at mortuary, AIIMS on whom medico-legal autopsy was conducted, were included. 5 ml of blood was collected and preserved in EDTA vials and brought to the toxicology laboratory to estimate the blood lead level using trace metal analyzer. Subsequently data obtained wasanalyzed by using appropriate statistical methods to estimate the blood lead level.

Exclusion Criteria

- All decomposed dead bodies.
- Unknown dead bodies.
- Dead bodies from other than south Delhi region and from outside of Delhi, although died at AIIMS and not from south Delhi jurisdiction.
- All the non medico-legal cases whose death occurred at AIIMS.

Preparation of Sample and Analysis

Instrumentation/Accessories and Operating Conditions

Voltammetric determination of Pb was performed by Trace Metal Analyzer model 797 VA Computrace from Metrohm AG Ltd, Switzerland (Figure 1). It is a three-electrode system consisting of Multy Mode Electrode MME (mercury) as working electrode, Platinum (Pt) as auxiliary electrode and Ag/AgCl/ 3M KCl as reference electrode. The operating parameters are given in Table 2. Nitrogen gas of high purity was used. Micropipette of Eppendorf make of volume 10-100 µl and 100-1000 µl were used. pH measurements were done with pH meter of model Inolab WTW series at ambient temperature of laboratory. Whatman filter paper 41 Ashless Circles of 125 mm Cat No 1441 125 from Whatman International Ltd Maidstone England were used.

Reagents/Chemicals

Suprapure acetic acid from Merck, Germany, Nitric acid, Liquor ammonia, ammonium oxalate, Lead nitrate from Merck Specialiaties Private Limited, Mumbai, sulphuric acid from Qualigens Fine Chemicals, Mumbai, ultrapure water from Rion India were used.

Preparation of Ammonium Acetate Buffer

Ammonium acetate buffer was prepared by dissolving 5.55 ml of acetic acid in 10 ml of ultrapure water. Then 3.7 ml of Suprapure ammonia was added slowly and pH was adjusted to 4.6 by adding few drops of Suprapure ammonia. Finally the volume was made upto 50 ml with ultrapure water.

Preparation of Standard Solution of Lead

1000 ppm solution of Lead was prepared from Lead Nitrate. 1 ppm standard of lead was prepared by diluting 0.1ml of 1000 ppm stock solution of lead to 100 ml ultrapure water.

Sample Preparation

Vessel of microwave digester was cleaned up by Nitric acid (HNO_3) and H_2O mixture (1:1) and dried. One gram sample was transferred into the linear vessels.15 ml of 35 % HNO_3 was added into each vessel and the mixture was left for few minutes for auto gas. In the reference vessel, 1 ml of water was added along with 15 ml of 35% HNO_3 for sample blank. Vessel carrousel was loaded in the microwave digestion oven and the digestion machine was run according to program given in Table 2.

After completion of run, microwave digestion was kept for cooling. After cooling, the vessels were opened and digested material was completely transferred in 50 ml volumetric flask with the help of ultrapure water and final volume was made upto 50 ml with ultrapure water.

Anodic Stripping Voltammetricmeasurements (Figure 1)

10ml ultrapure water and 1ml of acetate buffer (pH 4.6) was taken in polarographic vessel and then the measurement was started under the given parameters (Table 1). After this voltamogramme of the 'blank' was recorded. 0.1 ml of prepared sample solution was added to polarographic vessel and then voltamogramme of the sample solution was recorded under the same conditions. After the sample voltamogramme was recorded, 0.1 ml of 1 ppm standard of lead was added twice and then voltamogramme of the standard was recorded. Finally the concentration of the metal was calculated

by linear regression method (standard addition) using following formula

$$\text{Final Result} = \text{Concentration} \times \frac{\text{Cell Volume}}{\text{Sample amount}} \times \frac{\text{Multiplier}}{\text{Divisor}}$$

Where, Multiplier = Dilution

Divisor = Sample amount taken for preparation

Results

This study was conducted to estimate the blood lead levels in post-mortem cases of South Delhi area that was brought to the Department of Forensic Medicine and Toxicology, AIIMS, New Delhi. Two hundred and fifty cases were studied as per the inclusion criteria during the period of March 2012 to October 2013. We found that the mean blood lead levels in South Delhi population were 14.28 µg/dl and range was 2.34 to 53.69 µg/dl. 15.2% of south Delhi population were having blood lead level >25µg/dl. This observation is lower when compared to the study conducted by Kumar S et al [10] at Rohtak, on 42 male volunteersin the absence of local industrial sources of lead with varying degrees of exposure to vehicular exhaust. Automobile workers were found to have the highest levels of blood lead (mean value 21.26 µg/dl) followed by roadside population (mean value 14.91 µg/dl).

Sokas RK et al [11] conducted a cross-sectional study on Lead levels in Maryland construction workers. 264 Maryland construction workers had mean values of 8.0µg/dl, ranging from 2 to 30 µg/dl. None were currently engaged in known lead work. Blood lead levels were significantly higher for the 124 who had 'ever' worked in demolition (8.8 µg/dl). The 58 workers who had workplace lead monitoring had higher lead levels (9.7 µg/dl). Blood lead levels increased with age, and cigarette smoking.

Shobha N et al [12] conducted a study at NIMHANS, Banglore described the phenotypic and electrophysiological profile in five male patients working in a battery factory who developed radial nerve neuropathy due to lead exposure. All patients had elevated blood lead levels that were in the toxic range but higher to the study conducted by Reynolds SJ et al [13] that blood lead level for painters and laborers were significantly higher than other occupational group. The probable reason for higher blood lead level in South Delhi is might be due to higher rate of lead level in the environment namely air pollution (Vehicle exhaust), occupation related hazards etc.

Also, we found that mean blood lead level(Table 3) in male (16 µg/dl) was significantly higher than the female (10.41 µg/dl). 19.6 % male and 5.2 % female were having >25 µg/dl blood lead level. This finding is similar to the observation of Medical Surveillance of Blood-Lead Levels in British Workers [14]. The probable reasons for this may be due to male population being exposed more to the lead pollution than female.

Fig. 1: Trace Metal Analyser
(Make- Metrohm AG Ltd, Model- 797 VA Computrace)

Fig. 2: Microwave digestion system
(Make-Aurora Instruments Ltd., Canada, Model- MW 680)

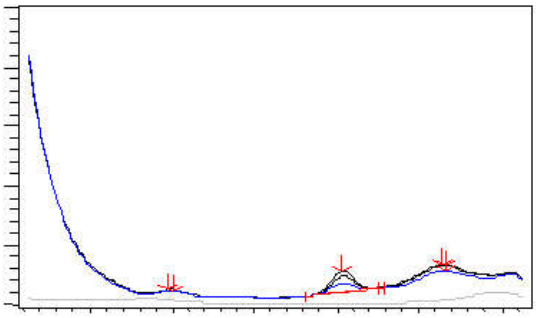

Fig. 3: DPAS Voltamogramme of Pb obtained from standard addition technique with number of replications being 2. A) 0.1 ml sample in 1ml acetate buffer (pH 4.6) + 10 ml distilled water, B) A + 0.1 ml standard solution of Pb (1 ppm), C) B + 0.1 ml standard solution of Pb (1 ppm)

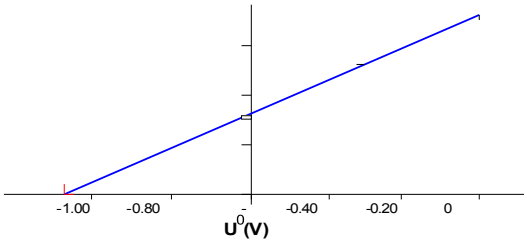

Fig. 4: Extrapolation graph of Pb obtained from standard addition technique

Table 1: Operating parameters for the determination of Lead

Parameters	Description
Working electrode	Hanging Mercury Dropping Electrode
Calibration	Standard addition method
Number of replications	2
Drop size	4
Stirrer speed	2000 rpm
Mode	Differential pulse
Initial purge time	300 s
Addition purge time	10 s
Deposition potential	-1150 mV
Deposition time	90 s
Equilibration time	10 s
Pulse amplitude	50 mV
Start potential	-1150 mV
End potential	-700 mV
Voltage step	6 mV
Voltage step time	0.1 s
Sweep rate	60 mV/s
Peak potential Pb^{2+}	-380 mV

Table 2: Programming conditions for the microwave digester

Step	Time (s)	Starting Temp (°C)	Ending Temp (°C)
1	210	28	100
2	600	100	160
3	600	160	170

Table 3: Mean values according to Sex

Gender	No of Persons	Mean value of Lead in µg/dl
Male	173	16
Female	77	0.41

Table 4: Mean values according to age group

Age Group in years	No of Persons	Mean values of PB in µg/dl
<14	8	7.28 µg/dl
14-17	18	8.07 µg/dl
18-40	172	13.71 µg/dl
41-60	40	20.73 µg/dl
>60	12	14.88 µg/dl

We also found in our study group that the age wise blood lead level (Table 4) was highest in middle age group and lowest in child age group. Mean blood lead level for middle age group was 20.73 µg/dl, range 3.84 – 53.69µg/dl and for child it was 7.28 µg/dl, range 2.34 – 17.42µg/dl. 50% children were having blood lead level >5µg/dl

Conclusion

Maximum number of cases occurred in the age-group of 18 - 40 years, followed by age-group of 40 - 60 years. Males outnumbered females with male to female ratio of 2.25:1. Mean blood lead level for South Delhi population was 14.28 µg/dl. Range for blood lead levels in South Delhi population were 2.34 – 53.69 µg/dl. Highest mean blood lead level was in middle age group 20.73 µg/dl. Lowest mean blood lead level was in child age group 7.28 µg/dl. Blood lead level for males were higher than females.

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