Original Article

Journal of Forensic Chemistry and Toxicology
Volume 5 Number 1, January - June 2019
DOI: http://dx.doi.org/10.21088/jfct.2454.9363.5119.7

Estimation of Zinc Levels in Blood, Liver and Stomach Contents using ICP-AES: A Cross Sectional Autopsy Based Study

Anita Yadav[1], Adarsh Kumar[2], Surender Singh[3], AK Jaiswal[4], Madhulika Sharma[5], Tabin Millo[6], SK Gupta[7]

Author Affiliation: [1]PhD Scholar [2,6,7]Professor [4]Chemist, Dept. of Forensic Medicine and Toxicology, [3]Professor, Dept. of Pharmacology, [5]Ex-Director, Forensic Science Laboratory, All India Institute of Medical Sciences, New Delhi 110029, India.

Corresponding Author: Adarsh Kumar, Professor, Dept. of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, New Delhi 110029, India.

E-mail: dradarshk@yahoo.com

Received on 02.04.2019, **Accepted on** 08.06.2019

Abstract

Zinc is an essential component of the body, but its excess quantity is harmful and long term intake above upper limits causes acute or chronic toxicity. Normal levels of zinc in serum is 75-120 µg/dL while in blood it is 1200 µg/dL. A study was conducted to determine the level of zinc in various biological samples from the medicolegal autopsies conducted at mortuary, Department of Forensic Medicine & Toxicology, All India Institute of Medical Sciences, New Delhi. Biological samples were taken from 100 cases comprising of Blood, Liver & Stomach contents from each case. They were analysed using Inductively Coupled Plasma-Atomic Emission Spectrophotometry (ICP-AES). The data obtained were analysed using various demographic profiles and treatment history to know prevalence & distribution of these metals in general population so as to help later in investigation of alleged deaths due to metal toxicity & metallic compounds poisoning. The result showed mean blood zinc levels were 14.21 µg/ml (range 0-77.36 µg/ml), mean zinc levels in liver and stomach contents were 25.66 µg/g (range 0.72–127.03 µg/g) and 7.95 µg/ml (range 0–60.94 µg/ml) respectively. The data analysis on the basis of treatment history showed that mean zinc levels in blood, liver and stomach contents were higher in cases where treatment was not given i.e. 14.97 µg/ml, 15.25 µg/g and 8.18 µg/ml respectively.

Keywords: Zinc; Autopsy; Blood; Liver; Stomach Contents; ICP-AES.

How to cite this article:

Anita Yadav, Adarsh Kumar, Surender Singh et al. Estimation of Zinc Levels in Blood, Liver and Stomach Contents using ICP-AES: A Cross Sectional Autopsy Based Study. J Forensic Chemistry Toxicol. 2019;5(1):37-41.

Introduction

Zinc is an essential component of the body, but its excess quantity is harmful it can be acute and chronic also. Acute adverse effects include nausea, vomiting, loss of appetite, abdominal cramps, diarrhoea, and headaches [1]. Intake of 150-450 mg of Zn per day is associated with chronic effects as low copper status, altered iron function, reduced immune function, and reduced levels of high density lipoproteins [2]. Chronically high intakes adversely affect some aspects of urinary physiology [3]. Hadla S. Ferreira et al. [4] in 2007 proposed a direct method based on slurry sampling of the determination of zinc and copper in human hair samples by multi-element sequential flame atomic absorption spectrometry. The slurries were prepared by cryogenic grinding and sonication of the samples. The optimization step was performed using univariate methodology and the factors studied were: nature and concentration of the acid

solution, amount sample/slurry volume, sonication time and particle size. The established experimental conditions were the use of a sample mass of 50 mg, 2 mol /l nitric acid solution, sonication time of 20 min. and slurry volume of 10 ml. adopting optimized conditions. This method allowed the determination of zinc and copper with detection limits of 88.3 and 53.3 ng/g, respectively, and precision expressed as relative standard deviation (RSD) of 1.7% and 1.6% (both, n=10) for contents of zinc and copper of 100.0 and 33.3 µg/g, respectively. The accuracy was checked and confirmed by analysis of two certified reference materials of human hair. The procedure was applied for the determination of zinc and copper in two human hair samples. The zinc and copper contents varied from 100-175.6 and from 3.2-32.8 µg/g, respectively. These samples were also analyzed after complete digestion in a closed system and determination by FAAS. The statistical comparison by t-test (95% confidence level) showed no significant difference between results. B Zerahn et al. [5] in 1999 conducted a study on thirteen soldiers (11 men and two women) who were exposed to zinc chloride smoke (ZCS) during a combat exercise. Even though their initial symptoms were modest, a prolonged follow up with lung function testing and blood samples was undertaken due to previous cases with fatal outcome after exposure to ZCS. Four weeks after exposure there were statistically significant declines from baseline values in lung diffusion capacity and total lung capacity of 16.2% and 4.3% respectively. At the same time plasma levels of fibrinogen and zinc were significantly elevated, though mainly within the normal range. All variables showed a tendency towards normalization at follow up 8 weeks and 6 months after exposure. These findings indicate an unexpected quantifiable damage to lung parenchyma with a remarkable delay after modest exposure to zinc chloride smoke despite sparse initial symptoms. JB Dawson et al. [6] in 1969 described a method for the determination of zinc in plasma diluted twenty fold with 0.1N HCl, in whole blood diluted one hundred times and urine diluted tenfold, using a Perkin-Elmer 303 atomic absorption spectrophotometer. Suppression of upto 15% of the apparent zinc content by inorganic components of the sample was overcome by the addition of the appropriate amounts of those ions to the standard zinc solutions used in the determination. The organic components of the samples had no significant effect on the apparent zinc content. Random contamination presented a problem which was best detected by replicate analysis. Studies of the plasma zinc level of 20 normal subjects (10 men, 10 women) showed a significant difference ($p<0.001$) between samples taken fasting at 9h and 5h later (14.00h) after the usual meals. The mean values were: 9h, men: 98 µg/100 ml, women: 96 µg/100 ml; 14h, men: 80 µg/100 ml, women: 83 µg/100 ml. the difference in whole blood values taken fasting at 9h and 5 hours later was not significant ($p> 0.6$) and the means of two samples were: men 584 µg/100 ml (range 414-794 µg/100 ml), women 582 µg/100 ml (range 342-700 µg/100 ml). The 24h urine excretions were men 585 µg (range 263 -817 µg) and women 414 µg (range 276-702 µg) this difference was significant ($p<0.05$).

Objective: To determine the level of zinc in blood, liver and stomach contents of post mortem bodies.

Material & methods

Chemicals and reagents: 69% Nitric Acid GR, 30% Hydrogen Peroxide, Ultrapure Water,

Biological Samples: Blood, Liver and Stomach Contents

Equipment used: Microwave Digester (MDS-10) from sineo company, ICP-AES (Inductively coupled plasma – Atomic Emission spectrophotometry) (JY2000) from Horiba Jobin YVON company.

Standard Preparation: Mix standard solution of zinc of 100 ppb, 500 ppb and 1000 ppb was prepared by diluting the 1000 ppm standard by using N1V1 = N2V2 Formula.

Sample collection: All samples (Blood, liver & stomach contents from each case) were collected from Mortuary, All India Institute of Medical Sciences, New Delhi.

Exclusion & Inclusion Criteria:

Inclusion Criteria: All medico legal autopsies without the history of metallic/metallic compounds poisoning.

Exclusion Criteria: All deaths with a history of metal/metallic compounds poisonings.

Procedure

Samples taken:

1. *Blood*: 10 ml of blood preserved in Sodium fluoride of 10 mg/ml and potassium oxalate of 30 mg/10 ml

2. *Liver:* 10 gms of liver preserved in saturated solution of sodium chloride (Common salt-36 gms/100 ml)

3. *Stomach Contents*: 25 gms stomach contents preserved in saturated solution of sodium chloride.

Digestion of sample

Various methods using different quantities of biological sample with varied reagent concentrations were tried but the valid sample composition is as follows:

0.5gms/ml of biological sample + 4.5 ml of 69% HNO_3 + 0.5 ml of H_2O_2

Sample loading

Prepared samples (total of 6 ml for each sample) were added to the vessels then the vessels were closed using a spencer. Vessel assembly was prepared and the vessels were put on the respective positions on the turntable inside the microwave digestion system by balancing the vessels.

Door was closed and digestion programme was started.

Microwave Digestion Programme

Various programmes of temperature ramping along with the sample composition (already mentioned) were tried among which the validated temperature programme is as follows:

Table 1: Programme showing ramping of temperature with respect to time

Temperature	Time in Minutes	Power(watt)
120	10	800
150	10	800
180	10	800
200	10	800

After completion of temperature programme the machine was left for cooling for 35 minutes. After cooling the door was opened and the vessels were taken out one by one with proper precautions. Vessels were then opened in the fume hood using the spencer and allowed to degas and then transferred to the tarson tubes of capacity 15 ml.

Fig. 1: Microwave Digestion System from Sineocompany

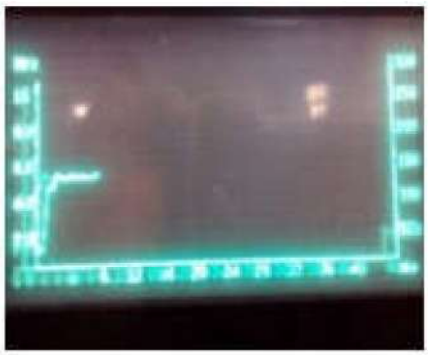

Fig. 2: Ramping of temperature programme shown in the form of graph on MDS 10

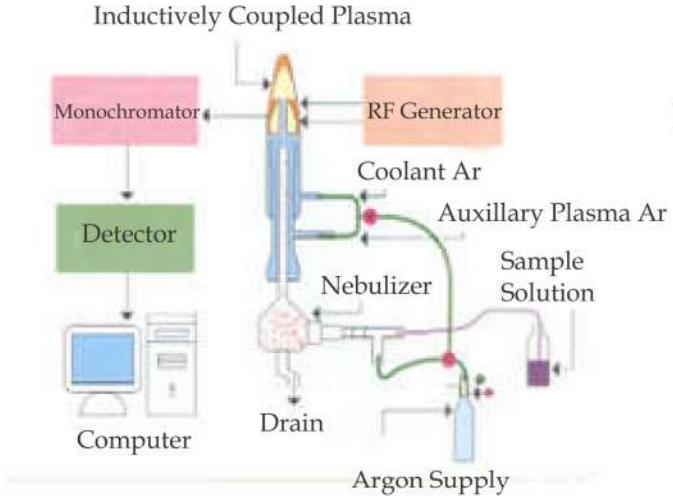

Fig. 3: Flow chart showing working of ICP-AES

Fig. 4: ICP-AES instrument

Analysis on Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP- AES) *[7]:*

ICP-AES is an emission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state after excitation by high temperature Argon Plasma. The fundamental characteristic of this process is that each element emits energy at specific wavelengths peculiar to its atomic character. The energy transfer for electrons when they fall back to ground state is unique to each element as it depends upon the electronic configuration of the orbital. The energy transfer is inversely proportional to the wavelength of electromagnetic radiation, $E = hc/\lambda$ ... (where h is Planck's constant, c the velocity of light and λ is wavelength), and hence the wavelength of light emitted is also unique. Although each element emits energy at multiple wavelengths, in the ICP-AES technique it is most common to select a single wavelength (or a very few) for a given element. The intensity of the energy emitted at the chosen wavelength is proportional to the amount (concentration) of that element in the sample being analysed. Thus, by determining which wavelengths will be emitted by a sample and by determining their intensities, the analyst can qualitatively and quantitatively find the elements from the given sample relative to a reference standard. The method for analysis of zinc was created as its peak was found on the wavelengths of 213.856 nm. The machine was calibrated using standard solutions of mix standard of zinc with different concentrations of 100 ppb, 500 ppb and 1000 ppb which were prepared by dilutions of 1000 ppm standard solution obtained from Merck. After calibration the samples digested were run for analysis and each sample was run thrice with three replicates of each. The parameters used for analysis of zinc is as follows:

Table 2: ICP-AES parameters for zinc detection

Parameters	Conditions
ICP-AES software version	5.2
Wavelength for zinc	213.856 nm
Plasma flow	12 L/min
Sheath flow	0-2 L/min
Pump speed	20 rates/min
Nebulizer flow rate	0.34 L/min
Nebulizer pressure	2.76 bar
Detector	Photomultiplier tubes

Results and Discussions

The data obtained were analysed using various demographic profiles and treatment history

Table 3: Average distribution of Zinc

Biological Sample	Blood (µg/ml)	Liver(µg/g)	Stomach Contents (µg/ml)
Mean Values	14.21	25.66	7.95

Table 4: Age wise distribution of Zinc

Range (Years)	Blood (Mean)	Liver (Mean)	Stomach Contents (Mean)
0-10	38.27	107.36	32.38
11-20	20.47	31.85	8.45
21-30	14.44	23.23	5.25
31-40	15.59	22.84	10.49
41-50	14.98	11.07	6.84
51-60	6.65	17.97	6.69
61 & above	10.72	17.89	14.89

Table 5: Sex wise distribution of Zinc

Gender	Blood (Mean)	Liver (Mean)	Stomach Contents (Mean)
Male	13.96	20.58	8.46
Female	15.7	17.16	5.67

Table 6: Distribution of mean values of Zincaccording to treatment history

Treatment history	Blood	Liver	Stomach Contents
Treatment history present	10.35	14.93	5.87
Treatment history not present	14.97	15.25	8.18

This study was conducted to estimate the blood zinc levels in post-mortem cases of South Delhi area that was brought to the Department of Forensic Medicine and Toxicology, AIIMS, New Delhi. Hundred cases were studied as per the inclusion criteria during the period of march 2014 tomarch 2016. We found that the mean blood zinc levels in South Delhi population were 14.21 µg/ml and range was 0 to 77.36 µg/ml. The mean zinc levels in liver and stomach contents were 25.66 µg/g with a range of 0.72 – 127.03 µg/g and 7.95 µg/ml with a range of 0 – 60.94 µg/ml respectively. Also on the basis of age-wise distribution the mean zinc levels were higher in the age group of 00-10 years i.e. 38.27 µg/ml and in liver and stomach contents also the mean zinc levels were higher in the same age group i.e. 107.36 µg/g & 32.38 µg/ml respectively.

On the basis of sex-wise distribution the mean zinc levels in blood was higher in females i.e. 15.7 µg/ml while in both liver and stomach contents it was higher in males i.e. 20.58 µg/g and 8.46 µg/ml respectively.

We also classified the data on the basis of treatment history which showed that mean zinc levels in blood, liver and stomach contents were higher in cases where treatment was not given i.e. 14.97 µg/ml, 15.25 µg/g and 8.18 µg/ml respectively.Normal levels of zinc in serum is 75-120 µg/dL and in blood is 1200 µg/dL [8]. The Food and Nutrition Board has established upper limits for Zn (table 7). Long term intakes above upper limits increase the risk of adverse health effects [1].

Table 7: Tolerable Upper intake level of zinc

Age	Male	Female	Pregnant	Lactating
0-6 months	4 mg	4 mg		
7-12 months	5 mg	5 mg		
1-3 years	7 mg	7 mg		
4-8 years	12 mg	12 mg		
9-13 years	23 mg	23 mg		
14-18 years	34 mg	34 mg	34 mg	34 mg
19 + years	40 mg	40 mg	40 mg	40 mg

Conclusion

This study was carried out to know the prevalence & distribution of these metals in general population so as to help in investigation of alleged deaths due to zinc toxicity and its related compounds poisoning. The findings also suggested that treatment can reduce the traces of zinc. Although zinc is an essential element but its excess levels also causes harmful effects that may be acute or chronic in nature.

References

1. Institute of Medicine, Food and Nutrition Board, Dietary reference intake for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington DC: National Academy Press, 2001.
2. Hooper PL, Visconti L, Garry PJ, Johnson GE, Zinc lowers high density lipo-protein-cholesterol levels. J Am. Med. Assoc. 1980;244:1960-1.
3. Johnson AR, Munoz A, Gottlieb JL, Jarrad DF. High dose zinc increases hospital admissions due to genitourinary complications. J Urol. 2007;177:639-43.
4. Hadla Ferreira S, Walter dos Santos NL, RaigenisFiuza P, Joaquim Nobrega A, Sergio Ferreira LC. Determination of zinc and copper in human hair by slurry sampling employing sequential multi-element flame atomic absorption spectrometry. Microchemical Journal. 2007;87(2):128-31.
5. Zerahn B, Kofoed-Enevoldsen A, Jensen BV, Molvig J, Ebbehoj N, Johansen JS, Kanstrup IL. Pulmonary damage after modest exposure to zinc chloride smoke.Respiratory Medicine. 1999;93(12):885-90.
6. Dawson JB, Walker BE. Direct determination of zinc in whole blood, plasma and urine by atomic absorption spectroscopy. ClinicaChimicaActa. 1969;26(3):465-75.
7. http://www.rsic.iitb.ac.in/Icp-Aes.html.
8. Haririson's Principles of Internal Medicine, Seventeenth Edition, 2008. Mc. Graw Hill Companies, Inc. Appendix: Laboratories Values of Clinical Importance.