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Spectrofluorophotometric Study of Zinc level in Biological Samples and its Comparison by Atomic Absorption Spectrophotometry

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The content of zinc in scalp hair of different age groups either sex of different socioeconomic value were determined by using Atomic Absorption Spectroscopy and Spectrofluorophotometer techniques. The level of zinc was high in female scalphair than those of male. The element of zinc was related to age 8 to 10 years and level of zinc increase with age in both sexes . The concentration of zinc was high in nails as compare to hair samples. The comparative study for zinc in hair and nail sample showed that Spectrofluorometric method was high value of zinc as compared to Atomic Absorption and this method applied to determine the micro amount of zinc in biological materials.

Key words: Hair, nail, biological materials

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Introduction

The determination of trace level of elements in biological samples is important in medical, environmental and forensic sciences (Chengfan *et al.*, 1990) . At present at least 17 elements are considered essential for human life. Their amount depends upon the food we eat and the atmosphere or the environment where we lived (Kazi *et al.*, 1999). Zinc is a part of 80 metalloenzyme found in all metabolic pathways (Golden *et al.*, 1988). The content of Zinc is an index of teratological defects observed (Schroeder *et al.*, 1987).

Analysis of hair and nails have been used as an indicator of exposure and utilization for several trace elements (Ophang, 1994) Metal content in hair and nails can be used in environmental monitoring and provides an additional assessment of the nutritional status groups of individual (Cantiero *et al.*, 1994). Both Atomic Absorption Spectrometry and Spectrofluorometry is now widely used techniques (Chatt *et al.*, 1988).

The research work was conducted to compare two methods to study metal ions in biological materials. Such data serves as a simple tool for monitoring exposure to potentially hazardous levels of these metals.

Materials and Methods

1. Atomic Absorption Spectrophotometer Hitachi 180-50.
2. Spectrofluorophotometer Schimadzu RF-510.

Hair and nail sample collection: Donor of hair and finger nail were local population of different localities of Hyderabad, Sindh, Pakistan.

Reagents: The following reagents of analytical grade were common to the AAS and SF i.e. 2N HNO_3, H_2O_2 and Zn pellets. Additional reagents used in the SF were:

1 5% 8 - hydroxyquinoline (oxine) solution in CH_3COOH.
2. Standard dichlorofluorescein solution, 0.1%.
3. 2% gums arabic solution.
4. Ammonium acetate and chloroform solution.

Procedure: Hair and nail samples were washed by method described Yaun Q and Libna (Yuan *et al.*, 1990). Fifteen washed, dried hair and nails sample were digested with conc. HNO_3 and H_2O_2 reported by (Kazi *et al.*, 1997).

Determination by Spectrofluorophtometer: To each 100 ml flask of Standard Zn, sample solutions of 10.0ml of the CH_3COONH_4 solution , 4.0 ml of the gum arabic and 0.4ml of the oxine solution were added with micrometer syringe and was shaken gently. This was then immediately extracted out in 100 ml of $CHCl_3$ and was transferred to the cell of a fluorimeter for measurement. Dichlorofluorescein solution was employed as a standard .

Determination by Atomic Absorption Spectrophotometer: First, blanks and standard solutions of Zn were read, followed by Zn in hair and nails samples and finally the internal standard to check percentage recovery.

Analysis: The concentration of Zn by both methods was calculated by statistical analysis performed on computer program QPRO4.

Results and Discussion

The amount of Trace elements in our bodies is not proportionately retained. Some trace elements are lost in feces, urine and perspiration, in hair and the skin (Kazi *et al.*, 1999).

Determination of Zinc in hair and nail samples by Spectrofluorometry: The results of analysis are given in Table 1 and 2 for hair and fingernail respectively. Values of Zn varied relatively widely among individuals, thus a sufficiently a large population should be analyzed if correlation is to be meaningful. The subjects were broken into five distinct age groups, each group is of 15 to 25 subjects.

Statistical Data for Standard samples of Zinc analyzed by SF and AAS

	AAS	SF
Concentration Range in ppm	0.00-0.250	0.00-0.125
Absorbance / % Fluorescence	0.00-0.053	0.00-136.5
Slope (m)	0.21	1090.29
Intercept (c)	0.001	2.0
Correlation coef. (r)	0.999	0.999

Keys: (AAS) = Atomic absorption spectroscopy
(SF) = Spectrofluorophotometer

Table 1: Estimation of Zinc (μg/g) in Scalp hair of local female population of Sindh by SF

	FN1	FN2	FN3
6 - 12 yrs	83.6 ± 7.2	103.5 ± 5.9	118.1 ± 9.4
12 - 18 yrs	137.8 ± 8.3	166.8 ± 9.6	175.9 ± 9.7
18 - 25 yrs	139.6 ± 10.1	171.1 ± 8.1	190.5 ± 7.9
25 - 45 yrs	174.1 ± 12.1	194.4 ± 14.8	232.6 ± 15.0
45 - 60 yrs	223.5 ± 9.6	229.3 ± 9.3	235.2 ± 9.3

Table 2: Estimation of Zinc (μg/g) in Scalp hair of local male population of Sindh by SF

Age Groups	MN1	MN2	MN3
6-12 yrs	91.1 ± 8.8	113.9 ± 9.9	115.3 ± 9.6
12-18yrs	115.0 ± 9.6	130.9 ± 9.5	161.7 ± 9.9
18-25yrs	127.2 ± 9.7	151.8 ± 15.7	177.6 ± 8.6
25 - 45yrs	169.5 ± 10.9	179.4 ± 11.0	210.4 ± 15.9
45-60yrs		218.2 ± 13.6	

Key:M = Male , N1 = Nutritionally Enriched
N2 = Nutritionally Average N3 = Nutritionally poor

Table 3: Estimation of Zinc (μg/g) in Nails of local population of Sindh by Spectrofluorometric technique

Age Groups (yrs)	No. of Obs.	Male (μg/g)	No. of Obs.	Female (μg/g)
6-12	15	837.1 ± 9.8	19	318.3 ± 9.5
12-18	18	774.4 ± 8.6	20	372.1 ± 6.9
18-25	18	689.6 ± 6.4	22	214.6 ± 4.5
25-45	20	626.3 ± 6.6	23	236.8 ± 3.6

Table 4: Comparison of Spectroflourometric (SF) with Atomic Absorption Spectrometry(AAS)

Sex	Age	Samples	No. of Obs.	Mean (μg/g) with S.D by AAS	Mean(μg/g) with S.D by SF
♂	6-12yrs	Hair	20	102.9±6.0	107.5±6.0
♀	6-12yrs	Hair	16	119.2±8.8	123.2±8.6
♂	12-18yrs	Hair	20	130.9±9.5	131.4±9.8
♀	12-18yrs	Hair	18	166.8±9.6	165.4±9.8
♀	25-45yrs	Hair	30	225.5±14.6	230.0±15.7
♂	25-45yrs	Hair	22	190.5±16.9	197.0±17.7
♀	25-45yrs	Finger nail	18	234.2±9.9	235.9±8.1
♂	6-12yrs	Finger nail	10	102.9±6.0	107.5±6.0
♀	6-12yrs	Finger nail	16	119.2± 8.8	123.2±8.6
♂	12-18yrs	Finger nail	16	117.6± 8.3	124.0±7.4

Table 5: Recoveries of Zinc by Spectrofluorometric and Atomic Absorption Spectrometric methods

	Zn				Zn		
Method	Found (μg)	Added (μg)	Recovery (%)	Method	Found (μg)	Added (μg)	Recovery (%)
AAS	125	122	97.6	SF	125	121	96.8
AAS	100	98.2	98.2	SF	100	97.1	97.1
AAS	200	199.7	99.8	SF	200	197.8	98.9

There are significant variations in the concentration of Zn in scalp hair that can be attributed to personal covariates such as age, sex and socioeconomic status. Zn content in hair increases with the increase in age. Maximum values of Zn for

hair was observed in the age group of 45 to 60 years. Values for Zn in scalp hair of female samples are relatively higher than for male samples of all ages. The tendency for hair from females to have higher levels for several metals than the hair of males may be related to the higher average inorganic(ash) content for hair from females (John *et al.*, 1975).
In case of fingernail, the inverse case was observed in age and sex related effects. The concentration of zinc in either sex decreases with the increase in age and Zn in male samples is relatively higher than females of the same age.

Comparison of Spectrofluorometric and Atomic Absorption Spectrometric method: Comparative study for Zn in biological materials was performed by Spectrofluorometric and Atomic Absorption methods on 175 subjects. (Table 3). Result in Table 4 showed that there was no large difference in results for concentration of Zn in hair and nail samples.The fluorescent complex of Zn with 8-hydroxyquinolinehave very low detection limit. The fluorescent determination of micro amount of Zn in scalp hair has the advantages of convenience and reliability as the method is specific and has less time consuming.

Precision and recovery: The percentage recovery test was performed by standard addition method (Table 5). The percentage recovery was good by Spectrofluorophotometric method as compared to Atomic Absorption Spectrophotometer for element excusively.
Seven hair and fingernail samples of 0.1g each were taken from one subject and Zn was measured. The result of 204 $\pm$ 4.5 ppm showed the precision of method and different amounts of Zn were added to 0.1g of hair and nail samples and recoveries were measured after wet acid digestion. The recovery of Zn was very good and greater than 90% by both of the methods.

Hair and Nail Correlation: Twenty five subjects of matched age and sex were selected to correlate Zn values in hair and nail but no correlation was observed. This also confirms the work reported by Vance (Vance *et al.*, 1988). Perhaps this could indicate that hair and nails do not incorporate internal trace elements in exactly the same way, despite their similarity in chemical composition It is indicated in Table 1 and 2 that Zn in nail is more concentrated than hair sample of same individual. The wet acid digestion with HNO_3 and H_2O_2 for sample preparation used, offered the advantages of minimum sample manipulation, was economical and did not create any pollution problem. The proposed fluorometric determination of micro amount of Zn in biological samples has the advantages of convenience, rapidity and reliability. The technique can be applied up to 50 samples per hour to determine micro amount of Zn in biological samples.

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