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Assessment of Zinc Status of School Going Children by Hair analysis Method (9-12 Years)

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Abstract: ***Zinc is an important micronutrient concerned with optimal growth, development and a healthy immune system. There is limited information on methods to estimate zinc deficiency using non-invasive methods so this study was carried out to estimate the prevalence of zinc deficiency among the school going children by hair analysis. A cross sectional study was carried out among the 100 school going children selected randomly. The samples were interviewed using questionnaires. Hair zinc analysis by flame atomic absorption spectrophotometry method in µg was conducted on a sub sample of 20%. , anthropometry (height in cms, weight in kgs) and 24 - hour recalls were conducted. There existed a strong positive correlation between dietary zinc consumption and hair zinc levels. Zinc status of majority (55%) of children was poor as assessed by hair analysis and it correlated well with dietary assessment of zinc status.***

Keywords: Hair zinc analysis, Dietary zinc assessment, Zinc deficiency

1. Introduction

Zinc as a micronutrient has been involved in a number of structural and biochemical functions at the cellular and subcellular levels, which includes enzyme functions, DNA and RNA metabolism, protein synthesis, gene expression, cell growth and differentiation and cell-mediated immunity [15]. Zinc is also needed for diverse physiological processes and metabolic functions including many aspects of the immune system, physical growth, reproduction and neurobehavioural development. The important sources of zinc include flesh foods, liver, fish, nuts, seeds, legumes. However the bioavalability of zinc from vegetarian diets is likely to be lesser than non-vegetarian diets as plant foods rich in zinc are also rich in phytic acid which is an inhibitor of zinc absorption [12]. As zinc is ubiquitous in the human body a number of biomarkers have been identified. In the 48 studies included in a review, a total of 32 potential zinc biomarkers were identified of which in healthy individuals plasma, urinary and hair zinc were reported to be reliable markers of zinc status[15].

Hair zinc concentration has been proposed as a useful index of longer-term zinc status [13].It has some advantages owing to its noninvasiveness, low cost and philosophical relevance of mineral balance. Hair mineral analysis does not measure the total body load of a mineral, but it infers information about the metabolism of minerals in the cells [11].Hair mineral analysis represents an average rate of mineral accumulation in the sample for over 2-3 months before sampling [25].

As zinc is involved in a number of bio-chemical and structural functions zinc deficiency can cause several health consequences especially among children primarily leading to stunted growth, as well as morbidity related to diarrhea, pneumonia, malaria and other chronic diseases. A recent epidemiological study by Akhtar has confirmed a high prevalence of zinc deficiency among South-Asian countries and in India the five-major states have reported an overall prevalence of 43.8 % among children belonging to low socio-economic groups. Given the nationwide prevalence of inadequate intake of zinc and its adverse consequences on the health of children the overall aim of this study was to assess the prevalence of zinc deficiency among the school going children in the age group of 9-12 years by hair zinc analysis and dietary assessment.

2. Methodology

A cross sectional study was conducted on the 100 school going children, studying in two government schools in Bangalore. Study subjects belonged to the age group of 9-12 years.

Inclusion criteria were to select subjects with no major clinical and medical complications and willingness to participate in the study. Human scalp hair samples were collected. Scalp hair samples, about 3-4 cm long were cut close to the scalp in the occipital region of the head using stainless steel scissors. The collected hair samples were immediately placed in cleaned polyethylene plastic bags, properly labeled, and transported to the laboratory. In the laboratory, the samples were sequentially washed with acetone and ethanol, and thoroughly rinsed with de-ionized water to remove pharmaceutical products, particulates, and other exogenous materials. The washed hair samples were subsequently dried in an oven at 90°C for approximately 20 minutes and stored in pre-nitric acid washed Teflon sample containers. Approximately 1.0 g of dried hair sample from each participant was accurately weighed and digested with 15 mL HNO3 in a digestion flask. Hair sample digestions were initiated at relatively low temperatures to prevent a violent reaction until all the hair samples were fully dissolved in the nitric acid. The temperature was then subsequently increased until the solution turns pale yellow to ensure complete sample digestion. The nitric acid digested hair samples were filtered into a 25 mL volumetric flask using a whatman filter paper (ash less) and diluted to the mark with de-ionized water. The nitric acid digested hair samples were subjected

to zinc analysis in a flame atomic absorption spectrophotometer using a pre-mixed burner and air-acetylene flame. Each sample was analyzed in triplicate and the averages of the zinc concentrations in the hair samples were calculated using the constructed calibrated curves [7]. The reference range was 70-400µg/g and values below 70µg/g were indicative of zinc deficiency[7].

2.1 Questionnaires/ interviews

The major tool used was questionnaire. A Standard predesigned extensive questionnaire was classified into two parts. The first part was the general information and the second part consisted of specific information aimed at assessment of zinc status.

General information: This section consists of personal information about the individual study subjects such as name, date of birth, socioeconomic status, parent's education level, occupation, number of siblings, birth order.

Diet and lifestyle related information: The second part of the questionnaire consisted of assessment of zinc status by anthropometric measurements (height, weight), 3 day 24-hour dietary recall method, food frequency questionnaires. Studies have shown that a combination of methods such as the food frequency questionnaires and 24-hour dietary recalls can be used to obtain more accurate estimates of dietary intakes than that of individual methods [23]. Also food frequency questionnaires provide valid estimates of nutrient intakes and could be used for dietary assessments [22].

Knowledge related to zinc deficiency: This section consisted of questions which were aimed to assess the student's knowledge regarding zinc. The section also included practice related questions such as food habits. The presence of common symptoms and illnesses such as tiredness, lethargy, presence of cough, colds, diarrhea, rashes, itching or eczema related to zinc deficiency were also assessed.

Physical activity related information: Physical activity pattern of the study subjects was assessed. The frequency of performing outdoor activities, duration involved in sports, preference of activity-outdoor or indoor, attitude towards physical activity was assessed. As studies state that negative attitudes to be a stronger predictor of physical activity than positive attitudes which may be an important target for intervention efforts to increase physical activity among children and adolescents[17].

2.1.2 Statistical analysis

SPSS version 18.0 software was used for analysis. The characteristics of the study subjects and outcomes were described as percent, mean, standard deviation (SD). Student t-tests and standard t-tests were used to determine the significance. Chi-square tests were used to find the association between zinc hair levels and age, gender, socio-economic status and dietary zinc intakes which determines if two categorical variables are related. The significance was defined at a level of 0.05.

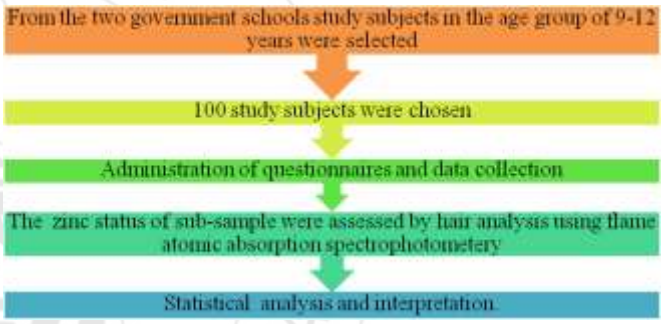

Figure 1: Flowchart of the study design and procedure

3. Results

Table 1: Characteristics of the study subjects:

Mean height and weight of male study subjects	Mean Weight (kg)	(n=40)	29.1 ± 1.2	30.8 ± 6.1	35.8 ± 4.8
	Mean Height (cm)		131.5 ± 6.1	143.1 ± 2.7	146.4 ± 4.6
Mean height and weight of female study subjects	Mean Weight (kg)	(n=60)	28.1 ± 9.8	30.5 ± 4.7	35.3 ± 3.9
	Mean Height (cm)		133.5 ± 5.9	142.1 ± 1.4	145.5 ± 5.7
Socio Economic Status	Upper Lower	%	87.5	84.4	53.5
		No.	28	27	30
	Lower	%	12.5	15.6	16.7
		No.	4	5	6
Sample (n)			32	32	36
Age group (years)			9-10	10-11	11-12

The table 1 shows the characteristics of the study subjects and for the classification of socio-economic status of the study subjects The Kuppuswamy socio-economic scale 2014 was used as a reference. A majority of the study subjects belonged to the upper-lower strata of the society. The mean heights and weights of the study subjects were below the 50th percentiles among all the age groups and genders when compared to the IAP growth charts (2015).

Table 2: Mean zinc intake of the study subjects

Gender	Age in years	Sample (n)	Zinc (mg)		Nutrient Adequacy Ratio
			RDA	Mean ±SD	
Male	9-10	12	8.0	2.2±0.5	0.275
	10-11	18	9.0	3.7±1.2	0.411
	11-12	10	9.0	2.4±0.9	0.266
Female	9-10	20	8.0	3.9±1.1	0.487
	10-11	14	9.0	3.3±1.2	0.366
	11-12	26	9.0	3.5±1.1	0.388

*Significant at 5% Level

The mean zinc intake was lowest (0.266) among the 11-12 year old males. The intake increased and then showed a decline among the male study subjects. Among the females the highest (0.487) consumption was among the 9-10 year olds and then there was a decline and an increase among the 11-12 year olds(0.388). NAR was calculated for each nutrient as the ratio of daily individual intakes to the standard recommended amounts for subject's sex and age category. The NAR was the highest among the females in the age group of 9-10 years (0.487).

$$NAR = \frac{\text{Daily nutrient intake}}{\text{recommended amount of nutrient}} \quad (1)$$

Table 3: Zinc hair levels of the study subjects

Gender	Age group years	Zinc hair levels				Correlation coefficient (r)
		< 70 µg/g		70-400 µg/g		
		No	%	No	%	
Male	9-10	2	100	0	0.0	+ 0.954
	10-11	2	50.0	2	50.0	
	11-12	3	75.0	1	25.0	
Female	9-10	0	0.0	4	100	+0.284
	10-11	2	100	0	0.0	
	11-12	2	50.0	2	50.0	
Combined	9-10	2	66.7	4	33.3	+0.264
	10-11	4	66.7	2	33.3	
	11-12	5	62.5	3	37.5	

NS: Non-significant

The correlation coefficients between age and zinc hair levels indicated a highly positive relation of +0.954 among the males and a much lesser value of +0.284 among the females and the overall positive correlation coefficient of +0.264.

4. Discussions

A strong positive correlation existed between dietary zinc consumption and hair zinc levels among the females compared to the males(r = +0.905, +0.882).A significant association of ($p<0.05$) socio-economic status and hair zinc levels was observed among the study subjects. Overall it can be said that there existed a strong positive correlation between dietary zinc consumption and hair zinc levels among the study subjects of the various age groups, genders as well as socio-economic strata. Overall the zinc status of majority (55%) of school going children was poor as assessed by hair analysis and it correlated well with dietary assessment of zinc status. Other studies have also found similar results and they can be summarized as

4.1 Gender wise variation among the hair zinc levels

A sub-sample of 20 children from the total sample size of 100 were selected for hair analysis by flame atomic absorption spectrophotometry. Females showed higher mean hair zinc levels (107.7µg) compared to males (78.5µg). Similar results were seen in the study conducted by [10] where females had greater hair zinc values than males. It is also evident that males had consistently lower hair zinc concentrations than females of the same age, even when food consumption patterns and energy and other nutrient intakes were comparable. Such sex differences in hair zinc concentrations may be because of higher zinc requirements for males possible due to growth hormone and testosterone concentrations [19]. The existence of a positive relationship between hair zinc status and growth percentiles has been noted earlier in the male pre-school children [24].

4.1.2 Correlation between dietary zinc consumption and hair zinc levels

A similar result was observed in our study was seen in the determination of zinc status using rats that hair zinc analysis could be used to aid diagnosis of a deficiency or evaluate dietary intake [5].

Hair as a tool for assessment of body load of minerals has been used in human and experimental studies. In human nutritional studies, especially for zinc and copper, head hair has been included. The concentration of zinc in human hair has been suggested to reflect the status of chronic zinc nutriture [9]. It was observed in one of the studies that the inherent characteristics of ease of collection and storage make it a parameter of choice in elemental (zinc and copper) studies [6]. The application of hair zinc concentrations as an indicator of zinc status has several advantages. Unlike serum zinc, concentrations of zinc in hair are more stable and is not affected by diurnal variation, prolonged fasting, meal consumption, and acute infection [3]. Furthermore once incorporated into hair zinc is no longer in equilibrium with the body and therefore not susceptible to circadian variation [6], [27].Unlike blood, hair samples do not need to be processed in the field and refrigeration is not required [13]. Association between low zinc concentration in hair and poor growth have been documented with reports linking low meat consumption or high phytate concentration with relatively low zinc concentrations in hair [8]. Hair zinc concentrations may be useful for tracking trends in zinc status over time within a population [13]. Data analyzed from 3 randomized control trails - supplementation studies, which included a total of 93 adult participants with either low or moderate baseline status and intakes suggested that hair zinc concentration was significantly elevated after supplementation thus indicating a reliable method for analysis [15].

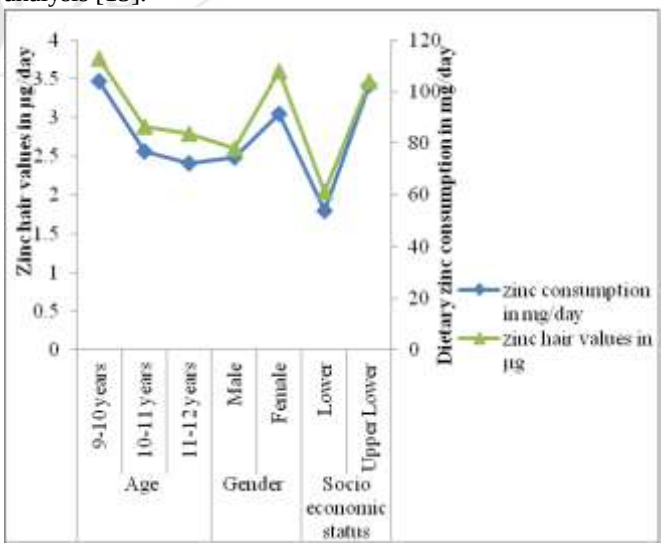

Figure 2: Correlation between hair zinc levels and dietary zinc consumption among the study subjects

5. Limitations

Collection of hair samples is less invasive than drawing blood and is more appropriate in some populations where collection of blood is not culturally acceptable, particularly from young children. The ease of collection and storage and processing is another advantage .A suitable nutrition education material was also designed to enhance zinc related knowledge.

A larger sample size would have powered to obtain a better accuracy for assessment of zinc deficiency. Due to time (6 months) and monetary constraints the hair zinc analysis could not be performed on a larger sample. The dietary data were self reported and were dependent on the students' recall and could have been subjected to recall bias. However a 3-day dietary recall was performed and pictures of food items and appropriate vessels were also used for dietary assessment.

6. Conclusions and Future Studies

Due to the lack of established cutoffs for most age groups and the uncertainties in interpreting results among malnourished children, the usefulness of hair zinc analysis in population zinc status assessment is presently limited. Hence studies involving direct estimation of zinc status using hair analysis need to be carried out as part of nationally representative nutrition surveys. As zinc deficiency affects the growth and immunity of children further research is needed to identify indicators of exposure to zinc deficiency as well as strategies to reduce zinc deficiency and its consequences.

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