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Elemental Profile and Hb Content in Whole Blood of Adolescents from Baramati Region, Pune, Maharashtra

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ABSTRACT

Levels of Ca, Mg, Zn, Fe and Cu were determined in whole blood samples of 147 adolescent participants aged between 13 and 16 years from Baramati region, Pune. The analyses were done using Atomic Absorption Spectroscopy and ICP-AES techniques. Haemoglobin content was analysed using Sahli's method. It is observed that 80% of the subjects suffered from various degrees of anaemia. From the Fe and Cu levels in whole blood, it was observed that 94 % and 81.9 % of the participants were deficient in iron and copper respectively. Zn, Mg and Ca levels were found to be higher than the reference range among 47.5 %, 54% and 59.5 % of the subjects. The obtained data was analysed using the software IBM SPSS Version 20. Fe and Cu showed significant correlation (r=0.353 at p<0.05). The study reveals that the subjects suffer from iron deficiency and will be prone to subsequent nutritional disorders.

Keywords: Major elements, Trace elements, SPSS, Haemoglobin.

INTRODUCTION

Major and trace elements play a crucial role in maintenance of human health and efficient metabolism. The levels of these elements in the human body must be monitored as excess or deficiency can result in ill-health. Food is the major source of intake of these elements. Ca and Mg are major elements play a role in bone health and is a co-factor in several enzymes [1]. Fe, Zn and Cu are among the essential trace elements [2]. Fe is associated with haemoglobin therefore, is important for oxygen transport. Its deficiency causes anaemia [3, 4]. Haemoglobin content in whole blood is an important marker in identifying anaemia [5]. Deficiencies of certain vitamins, Ca, Cu and sometimes excessive intake of Zn can also lead to anaemia. Zn is a constituent of DNA and several enzymes such as RNA polymerase and superoxide dismutase. Its deficiency is known to hamper physical development, wound–healing and DNA synthesis among children. Studies have shown that Zn toxicity can lead to deficiency of Cu. Cu is a part of metabolism of Fe and energy. It is required for development of nerve-coverings, bone and connective tissue. Deficiency of Cu leads to anaemia and affects immunity [3]. It has been observed that certain elements affect the levels of certain other elements in the body. Therefore, it is important to study various groups of population to identify prevalence of deficiencies of some major and essential trace elements to prevent disorders caused by deficiencies. The bioavailability and absorption of these elements in the body are influenced by levels of other elements [6, 7, 8, 9]. The complex interactions among the elements with each other may result in deficiency or excess of a particular element. For example, Zn and Cu compete for intestinal absorption. Therefore, high Zn levels can inhibit Cu absorption and lead to Cu deficiency [10]. Hence, the role each of these elements play in the body are rather complex.

Among the high-risk groups of insufficiency of these elements are adolescents. Adolescents are unique in the sense that they are different from adults and children in terms of higher nutritional requirements. Adolescence is a period of rapid growth, both physical and mental. This group of the population is at a high risk of deficiency of certain elements as the quantity of intake of nutrients does not fully meet the requirement [11]. For example, Fe requirements increase during adolescence due to growth spurt and puberty. Therefore, adolescents are prone to iron deficiency and subsequent nutritional disorders.

This study is important in the view that no such data is available for adolescents from this geographical region. AAS and ICPAES are one of the most popular methods for analysis of elements of our interest, owing to their properties of sensitivity, specificity, accuracy and cost-effectiveness. In the present study, we have analysed Mg, Fe, Zn and Cu levels in whole blood of 50 adolescents using ICPAES & Flame AAS techniques. Hb was analysed using Sahli's method. The subjects were chosen from Baramati in Pune district, India. The associations between these essential elements and prevalence of deficiencies of these elements have been investigated in this study.

MATERIALS AND METHODS

Subjects: A group of 147 school children aged between 13-16 years from Baramati, Pune district were randomly chosen for sampling. All the subjects and their parents were inhabitants of Baramati region, Pune district. The subjects belonged mostly to the lower middle-class strata.

Sampling: Informed consents were obtained from the parents of the children whose blood samples were collected prior to blood collection. After disinfecting the skin, 3mL intravenous blood samples were collected from the participants using disposable syringes. The blood samples were collected in BD-EDTA vacutainers and stored at -18^{o}C until further analysis. Information about their dietary habits, source of drinking water, symptoms of ill-health, if any, physical activities etc., was also collected using a semi-structured questionnaire.

Haemoglobin and Elemental Analysis: Haemoglobin content was analysed within 24 h of sampling by Sahli's method. 3 mL of blood sample was digested with equal volume acid mixture (nitric acid, sulphuric acid and perchloric acid in the ratio 3:1:1 respectively) at 150^{O}C for 2.5 h. All glassware were thoroughly cleaned using dilute nitric acid and rinsed with deionized water before use. Extreme care was taken to minimize contamination. The digested samples were diluted subsequently using deionized water for elemental analyses. The elemental analyses were carried out using ICPAES facility (Model: ARCOS, Simultaneous ICP Spectrometer; IIT, Mumbai) for iron and Flame AAS (Model: Schimadzu AA 7000) for Cu, Zn and Mg. Flame AAS technique is more cost effective in assessing trace elements in whole blood as compared to other sophisticated techniques [12].

Chemometric Analysis: Data was analysed using the statistical package SPSS (IBM SPSS 20). Descriptive statistics viz., Mean, standard deviation and median were computed for the elemental data. Correlations between the different elements were studied using Pearson correlation test with p value < 0.05 as significant. The dimensions of the data were determined using Principal Component Analysis. A cluster analysis was done using Ward's linkage and dendograms were plotted.

RESULTS AND DISCUSSION

The adolescent children hailed mostly from lower middle-class section of the society. 50% of the participants were male. Information about the parents' education status revealed that 13% of the participants' parents were illiterate, 35% had received primary-level formal education, 43% received high-school level education and 4% were graduates. 79% of the participants consumed non-vegetarian diet. BMI status was calculated for the participants. It was found that 34.2% were underweight or suffering from chronic energy deficiency, 50% had normal BMI status and 6% were found to be overweight.

Elemental Analysis: The age and range of Cu. Fe, Zn, Mg and Ca levels in whole blood are summarized in table 1.

Table 1. Descriptive statistics of the elemental concentrations in whole blood; N= 147

	Min	Max	Mean	Median	Std. Deviation
Age (y)	12	16	13.74	14.00	0.93
Cu (mg L^{-1})	0.16	3.15	0.70	0.65	0.29
Fe (mg L^{-1})	76.61	451.70	289.01	279.98	68.76
Zn (mg L^{-1})	3.07	19.67	9.02	8.80	3.27
Mg (mg L^{-1})	5.76	116.70	46.44	44.60	20.49
Ca (mg L^{-1})	43.58	93.02	70.81	68.78	10.44

Cu, Zn, Fe, Mg and Ca are the essential trace elements studied in this work. The interpretation of the obtained results is dependent on the reference intervals. A few studies have reported reference intervals of the blood levels of elements of our interest for adolescents. The reference intervals chosen for the interpretation of these results are shown in table 2.

Table 2. Reference intervals for various essential elements in whole blood of adolescents

Element	**Reference intervals**
Copper/ (mg L^{-1})	0.8 -1.30 [13]
Zinc/ (mg L^{-1})	4.4 – 8.6 [14]
Iron/ (mg L^{-1})	400 – 600 [14]
Magnesium/ (mg L^{-1})	28 – 40 [14]
Calcium/ (mg L^{-1})	46 – 62 [14]

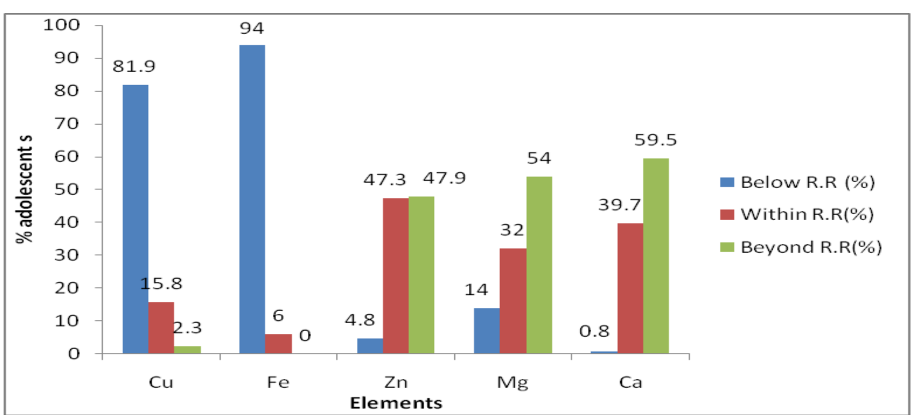

Fig.1. Chart showing percentage of adolescent subjects having blood elemental levels below, Within and beyond Reference range (R.R).

From the bar chart in fig.1 it can be seen that 94% and 81.9% of adolescents have blood levels of Fe and Cu lower than the respective reference ranges. This indicates a high prevalence of Cu and Fe deficiency among the adolescent participants. However, 14% adolescents were found to have insufficient blood Mg levels and 4.8% of the subjects had lower levels of blood Zn. 59.5% of the participants were found to have blood Ca levels beyond the reference range while 54% were found to have excessive levels of blood Mg.
Iron deficiency is the most common nutrient deficiency observed among adolescents [11]. Also, a notable finding in our study is that 98% of the participants with insufficient Cu levels showed Fe insufficiency. The mean elemental levels sex-wise are shown in table 3. High intakes of Zn are said to act as inhibitor in Cu absorption. This may be one of the factors leading to Cu insufficiency [15, 16].

Table 3. Sex-wise means of different elemental levels among adolescent participants

	Male (n=74)	Female (n=73)
Mean Cu/ (mg L^{-1})	0.7	0.65
Mean Fe/ (mg L^{-1})	294.60	284.04
Mean Zn/ (mg L^{-1})	8.03	9.04
Mean Mg/ (mg L^{-1})	45.58	47.71
Mean Ca/ (mg L^{-1})	70.34	71.31

The mean Cu levels and Fe levels are lower in females than in males. A plausible reason for this variation is the increased demand for Fe among females However, mean Zn levels, Mg levels and Ca levels are higher among females. Males require more Ca and Mg during adolescence due to accelerated bone and muscle development when compared to females. Therefore, inadequate intakes of Ca and Mg among males may be the cause for lower mean Ca levels. Significant differences between Zn and Mg between sexes have been noted in literature. The mean value of Zn was 9.047 mg/L for females and 8.034 mg L^{-1} for males which is much higher than most of the reported values for blood Zn. However, one report shows that the upper value in the reference range of Zn in Indian females as 9.2 mg L^{-1} [15]. Considering this as the upper reference value for blood zinc, the % adolescents having excessive blood Zn levels reduces from 48% to 42%. During adolescence, zinc retention increases considerably as a result of increased demand. This could be a possible reason for the elevated blood zinc levels among adolescents. The common routes of exposure to Zn in a population are through pesticide use, food additives, supplements, medicines, disinfectants, antiseptic preparations, talcum powder and other cosmetics. So far, there is no evidence of any significant health hazard owing to usage of zinc containing products. The extent of risk of toxicity linked to these exposure routes is a subject of debate in the annals of toxicity and human health [17].
The Haemoglobin content of the adolescent subjects ranged between 7.5 g dL^{-1} and 15 g dL^{-1}. The mean Hb of the study participants was 10.67 g dL^{-1}. Among these, 25% were mildly anaemic, 51 % were moderately anaemic, 4% was severely anaemic and 20% were non-anaemic as depicted in fig.2. This is indicative of high prevalence of anaemia among the adolescents in this region.

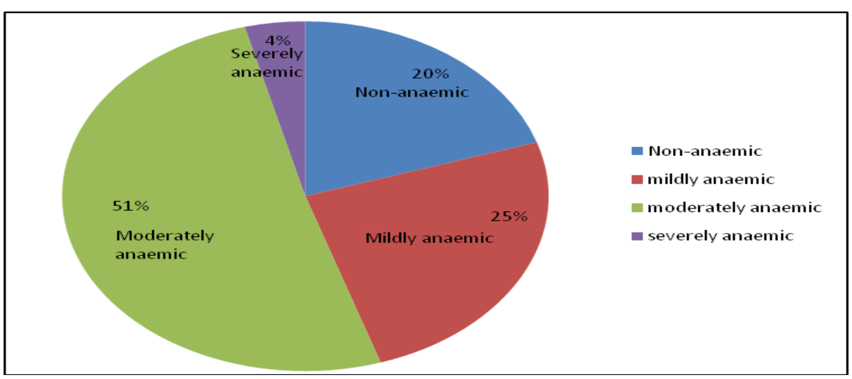

Fig. 2 % Prevalence of anaemia among adolescents (n= 147)

67 % of the adolescent participants who were insufficient in Cu and 78.2% of the participants who were insufficient in Fe were found to be anaemic. This suggests that Cu insufficiency and iron deficiency are one of the main factors responsible for anaemia among the participants. [18]

The Correlations between the elements were studied using Pearson correlation coefficient. The Pearson correlation coefficients are shown in the table 4

Table 4. Pearson correlation matrix of different elements; n=147

	Fe	Cu	Zn	Mg	Ca
Fe	1				
Cu	0.087	1			
Zn	0.075	-0.056	1		
Mg	0.233*	0.041	-0.031	1	
Ca	0.102	-0.030	0.038	0.210*	1

From table 4, it is evident that there are significant positive correlations between Fe and Mg with Pearson correlation coefficient being $r = 0.233$ at $p < 0.01$ and Ca and Mg with $r= 0.210$ at $p<0.05$. There were minor positive correlations seen between Fe – Cu, Fe – Zn and Ca – Zn. There were minor negative correlations observed for Cu – Zn and Cu – Ca.

Ca and Mg metabolism are closely related as their absorption pathways are inter-linked. Zn has an antagonistic effect on Cu metabolism.

Also, no significant correlation ($r = 0.129$) was observed between Fe levels and haemoglobin content in the blood. Similar observations were found in a study on iron and haemoglobin status involving working women of various age-groups. [5]
Cluster analysis was done and dendograms (Fig 3) were plotted based on the analysis. Cluster analysis is a very useful technique in identifying indirect patterns in Chemometric analysis. In our case, the parameters (Cu, Zn, Mg, Ca and Fe) are grouped based on their similarities. The analysis was carried out using Euclidean distance and Ward's linkage method.

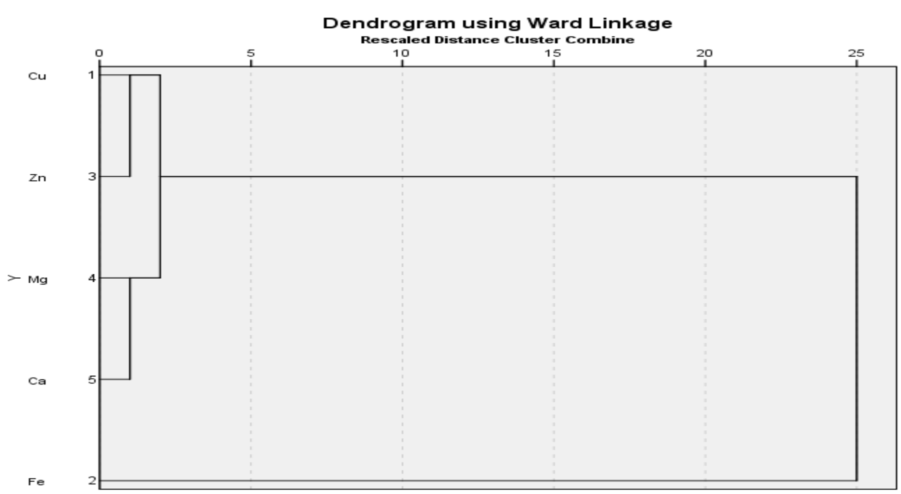

Fig 3. Dendrogram for Cu, Zn, Mg, Ca and Fe levels in adolescents

The clusters observed in Fig 3.
Cluster 1: Cu with Zn; Cluster 2: Cu with Mg; Cluster 3: Mg with Ca; Cluster 4: Fe with Mg and Cu.
The vertical distances are minimal in Clusters 1 and 3. Cluster 3 suggests there is high similarity between Mg and Ca. From table 4, it was seen that the levels of Ca and Mg significantly correlated. Cluster 4 suggests that Fe is correlated with the cluster 4 (Cu and Mg). Fe levels are thus correlated with Cu and Mg. Significant correlation was observed between Fe and Mg levels. Thus, clusters formed further substantiate the correlations observed in the Pearson's correlation table. 4.

Principal Component Analysis: This tool is very useful in reducing the dimensions of the data. Table 5 shows the principal components and the coefficients. The principal component analysis resulted in two principal components. The first principal component encompasses maximum variation in the data possible. [19] The second, third and fourth components represent as much as the remaining variation as possible. From Table 5, it can be seen that the loadings for Mg, Fe and Ca (0.741, 0.666 and 0.587 respectively) are large in the first principal component. It suggests that these parameters contribute largely to the variation. In the second component, large loadings are observed for Cu and Zn (-0.737 and 0.660 respectively). Thus, the five variables have been reduced to four components which have accounted for maximum variance (85% of the total variance).

Table 5. Table displaying the component matrix of two principal components

Elements	Principal Components			
	1	2	3	4
Cu	0.172	-0.737	0.440	0.478
Fe	0.666	-0.074	0.376	-0.455
Mg	0.741	-0.085	-0.228	-0.186
Zn	0.105	0.660	0.674	0.209
Ca	0.587	0.289	-0.389	0.573

The loadings for Cu and Zn are comparable in magnitudes but in opposite directions. This shows that as the variance owing to Zn levels is increasing in the direction of this vector in the second component, the variance from Cu levels in that direction is decreasing and vice-versa. Thus, increase in Zn values corresponds to the decrease in Cu levels.

MANOVA test was performed using R language. Significant differences (F = 0.02696) was observed in Fe, Cu and Ca across the BMI status of the adolescent participants suggesting that BMI status maybe influencing the levels of Fe, Cu and Ca in whole blood of adolescents. Significant difference was observed in Zn levels across the gender of the participants. Zn levels in whole blood are influenced by the gender of the participant as seen earlier in the discussion.

APPLICATIONS

The techniques employed in the determination of the elemental profile (AAS & ICP-AES) are cost effective and highly efficient. The findings of this study are crucial in understanding the nutritional status of the adolescents from this geographical region as no known studies have been performed to assess the elemental profile of the adolescents from this area. This study thus provides an insight into the vulnerability of adolescents to development of nutritional disorders. These results can be useful for designing intervention programmes for adolescents to reduce the prevalence of various nutrient deficiencies.

CONCLUSIONS

As indicated by the results, the subjects in the area are suffering from anaemia and subsequent nutritional disorders. The problem must be addressed through creating awareness on importance of nutrition and healthy dietary practices. Highest % insufficiency was observed for Fe (94%) followed by Cu (81.9%) and Mg (14%). Levels of Zn, Mg and Ca were found to be higher than the reference range among 49.5%, 54% and 57.9% of the subjects respectively. Chemometric analysis of the data was done by studying Pearson correlation, cluster analysis and Principal component analysis. Strong correlation was observed between Fe and Mg with r=0.233 and Ca and Mg with r=0.210. Cluster analysis resulted in 3 primary clusters and one secondary cluster. The principal components analysis resulted in four components based on the variances. MANOVA test suggested that BMI levels of the adolescents had an influence on Fe, Cu and Ca. Thus, correlations and trends of variance among the elements analysed are observed from the Chemometric analysis.

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