Original Article
Trace Element Indices in Hair and Saliva of School Children

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ABSTRACT

Evaluation of essential trace elements in hair and saliva is gradually being accepted as a useful tool in both scientific research and the diagnoses of disease. The normal concentration of these trace elements in hair and saliva; iron, zinc, copper, manganese were determined in 265 healthy children (7-9y) and their relationship to the health and the learning ability of the children were also investigation. The concentration of these trace elements in hair and saliva were in (Hair: Fe 28.47±0.70mg/kg; Zn 172.08±4.49mg/kg; Cu 21.03±0.79kg; Mn 1.30±0.05kg and Saliva: Fe 1.06±0.03mg; Zn 0.64±0.39mg; Cu 0.19±0.09mg; Mn 0.11±0.00 nmol/L). There was a significant high levels of trace element in hair but reduced concentration in saliva samples when compared with the reference means (P<0.05 in each case). There is a strong correlation between Zn concentration in saliva and Zn concentration in hair (P<0.05). Also there is a significant relatively low saliva Mn levels compared with the proportion that meets the thresholds (0.11±0.003nmol/l). In comparing samples with same parameters there was no significant correlation in most of the parameters. Anthropometric data and socioeconomic status had no effect on the levels of these elements in hair and saliva. The results indicate the possibility of assessing the presence of these elements using non-invasive methods in the absence of contamination and substantiate the potential of hair and saliva as a biomarker but could not ascertain the exact tolerable levels of the elements.

Key Words: Trace Elements, Children, Hair, Saliva and Ceres.

INTRODUCTION

Hair and saliva has become one of the most valuable and effective tools in analyzing trace elements status in human[1]. It can help to facilitate the identification of any abnormities be it toxic or deficiency, thereby helping in identifying the possible immune status of children. Information on the levels of trace elements in biological specimens in children is rear, especially in sub Sahara Africa[2]. At present, the use of trace elements concentrations in plasma or serum is widely accepted as norm but same can not be said of other tissue indices; hair, saliva, urine and nails.[3,4,5] The use of multi-element analysis to assess an individual's nutritional health or predisposition to disease has been controversial, but more studies which correlate concentrations of essential elements in organs like hair to deficiencies in tissue and body levels, has made it more acceptable due to

more evidence is now available which document the presence of toxic elements such as Arsenic, Cadmium, Lead and Mercury in hair to excess exposure, and serves as a useful diagnostic index of toxic elements.[6,7,8] Experts now agree that diagnosis and prevention of disease using saliva assay is possible due to steady progress made over years. Prompting more institutions, laboratories and medical practitioners to be involved in various researches using saliva, thereby making it more acceptable and due to the convenience and easy of collection, no pains involve during collection like the use of blood[9]. This technique (noninvasive) stands out as an important tool in assisting the determination of trace elements status, especially in rural communities that might not afford the invasive method or as a prerequisite for the use invasive method. However, research has shown that the human body cannot synthesize trace elements as

in case of copper, where the human diet must supply regular amounts for absorption[10]. Excess copper lowers zinc levels and produces hair loss, insomnia, depression and schizophrenia, while low copper levels are associated with Menke's and Wilson's disease[11]. Copper deficiency, in turn, is known to provoke iron deficiency and anemia. Zinc, a nutritionally essential element, may influence both the absorption and the toxicity of lead[11]. The interactions between environmental lead and environmental zinc levels and blood lead concentrations suggest that zinc may influence the association between soil and dust lead and corresponding blood lead levels.[12]

Hair copper usually reflects tissue levels except in copper transport diseases such as Menke's and Wilson's disease, which may present with low copper concentrations and is mostly associated with water leached from copper pipes, and from certain dyes and hair bleaching agents[11]. Zinc is an enzyme and is essential for most metabolic pathways, and should be available in reasonable amount likewise iron and manganese as they are essential for metabolic activities and physiological functioning of the human body[13]. Hence, the knowledge of trace element levels is very essential in the growth and development of children. It has been scientifically proven that hair trace mineral analysis is useful for the evaluation of person's general state of nutrient and health, and has been confirmed in most studies, including these study also it is valuable in detecting predisposition to disease and will assist the Doctor in determining if a patient is suffering from mineral deficiency or mineral in-balance or heavy metal pollutants in the body which may be responsible for a particular illness[14] and to establish an understanding between essential trace elements in hair and the same elements in organs[15]. Trace elements deficiency may not affect learning ability directly but may affect children through its impact on resistance to infection and in turn affect their school attendance and subsequent poor learning ability[16], as indicated in this study, it will best address the course and discrepancies associated learning difficulties.

METHODOLOGY
Study design
This pilot study aimed at assessing the levels of iron, zinc, copper and manganese in hair and saliva (noninvasive). This technique is widely used for the determination of trace elements in matrices, especially biological materials, and making them useful for analyzing iron, zinc, copper and manganese in grade one learners in primary schools in Ceres, Western Cape, South Africa. Prior to the data collection parents of the children under investigation were asked through questioners on whether special shampoos, relaxers or cream were used on their hairs, and also if they had any direct contact with objects that might increase their venerability to these elements contamination. Where the answer is yes, the samples collected were discarded and not included in the analysis.

Study population and study sample
The study population consisted of all grade one learners attending the six primary schools in Ceres in 2003 and 2004. The study sample was selected randomly from each school, using random number tables, with proportionate representation of each school. A total number of 265 learners were included in this study (Table 1) following informed consent, 62% of those asked to participate, signed consent forms.

Data collection
Sample and data collection took place during school hours over a period of one week towards the end of each year. Samples were prepared for analyses within one week of sample collection.

Determination of iron, zinc, copper and manganese in hair and saliva samples
The determination of trace elements (iron, zinc, copper and manganese) in hair and saliva, using the conventional *aqua regia* digestion procedure, consist of dissolving of samples in a 3:1 mixture of HCl and HNO_3 and digested in a hotplate for about 3hrs[17]. A photometric method was used in analyzing the digested samples using atomic absorption spectrophotometer (AAS) (Unicam AAS Type solar)[18,19].

Spectrophotometry: About 5mL of digested and made up samples was analyzed using the AAS machine at a wavelength most suitable to the particular element been analyzed with minimum or no interference. Precaution was taken throughout the experiments to avoid contamination of the samples, reagents and chemicals used. The samples were weighed accurately. Extra care was taken to avoid errors in reading coursed by acid interference, common with *aqua regia* method. In order to obtain reproductive results, it was important that we maintain constantly optimal aspiration and furnace condition. All machine readings were repeated twice.

HAIR: Approximately 0.5g of hair, obtained mostly from the back of the head close to the neck, was obtained from each learner using a sterilized stainless steel scissors. The scissors were cleaned with surgical spirits after each hair collection.

Hair samples were then washed with non-ionic detergent and rinsed with distilled water, oven dried for four (4) days at 60-70°C and stored in an airtight plastic bag. A well-mixed 0.25g of dried hair was weighed into a beaker and digested in 12mL of *aqua regia* (1/3 HNO_3 and 3/4HCl) acid, heated in a Gerhardt (Trace metal digestion units, DIN 38414) heated digestion block. The maximum digestion temperature was 120°C and to avoid loss of materials each beaker had a glass lid. Digestion continued until a clear and almost colorless solution was obtained. Each sample took 2-3 hrs to digest. The clear solution obtained was allowed to cool, filtered with Whatman no. 42 paper and diluted to a final volume of 100mL with di-ionized distilled (DDW) water[20]. This solution was stored in a plastic container until analysis using AAS was performed.

SALIVA: At least 5.0mL of mixed saliva was collected from the learners into a detergent washed polypropylene vial by direct spouting collection. The samples were checked for food and blood or nasal discharge contamination and contaminated samples were discarded. Four samples that did not have matching hair samples were discarded. The mixed saliva was then frozen and stored in a freezer at 0°C to 4°C.

Prior to the sample preparation, the saliva samples were defrosted and allowed to equilibrate to room temperature before being rechecked for any trace of contaminants. Five mL of saliva was then measured into a beaker and 20mL of 2% nitric acid (HNO_3). This solution was then filtered with Whatman no. 42 filter paper into a measuring cylinder, diluted to a final volume of 100mL with DDW[20]. The 100mL solution was then stored in a plastic container until analysis with AAS for zinc, iron, copper and manganese was done.

Ethical considerations
The Senate Research committee of the University of the Western Cape provided ethical approval for this study (SHD of 2004/6). The participation of learners was voluntarily following informed consent by parents or guardians. The participants were free to terminate participation at their convenience. Confidentiality of the data collected and subsequent findings were assured by using only code numbers for each participant.

Statistical analysis:
The data were analyzed using SAS version 8.12[21]. The results are presented as mean, standard deviation, in all samples, correlation coefficient (Pearson Correlation Coefficients) between zinc, copper in hair and saliva, also manganese in hair and saliva. The P-values <0.01 was considered statistically significant.

Results:
Table 1 shows the selection process, sixty-two percents of study samples consented to participate in the experiment. Representing 265 of the total 426 grade one learner over the study period (Table 1) with a male: female ratio 1:1, at an average age of learner was 7.73±0.60yrs. The average weight and height of the learners were 21.93 ±4.8kg and 118.69 ±7.2cm, respectively. The median household income contributor were 2 persons and that of income was R250-R99 per month (Table 2).

Table 3 shows the total number of samples collected from each participant, the average levels of zinc, iron, copper and manganese the hair and saliva samples and their reference values. The average concentration of all elements in hair include; Hr_Zn 172.08mg/kg with a standard reference of 150-250mg/kg and 95% of samples below the reference, Hr_Fe is 8.47mg/kg with the reference 6-15mg/kg and 86% of samples are within the reference, Hr_Cu 21.03mg/kg the reference is 15 – 35mk/kg and 60% within the reference and Hr_Mn 1.30mg/kg is high when compared with reference (0.2 - 0.8mg/kg) and 95% above the reference values. The saliva showed a contrary results to that of hair with Sl_Fe 1.06mg/l and the reference 1.52 - 5.72mg/l, 94% of values are below the reference, similar results can be found with Sl_Cu 0.20mg/l and the reference 1.26 – 2.99mg/l likewise Sl_Zn 0.64mg/l and reference 0.5 – 1.20mg/l with 52% of samples below the reference.

When compared with the standard value of ≤1mg/kg and contrary result was observed in saliva with most of the samples concentration are within reference value in both iron and copper (Sl_Fe 1.06±0.03mg/l and Sl_Cu 0.19±0.09mg/l) with the standard been Fe 1.52 – 5.72mg/l and

Table 1: Participants Data One: Number of participant and their percentages over the two Phases.

S/N	School Code	No. Of pupils in the school	Number of participants	Number of participants	Percentage of participant	Ration of Girls/Boys
Phase one		Learners	Selected	Consent	Response rate%	(M/f)
1	November. 2003	544	200	120	60.0	43/57
2	September 2004	688	226	150	64.5	54/46

Table 2: Demographic and Socio-economic data of learners

		Age of participants	Weight	Height	Family members contributing to household income	Family average wage
Phase One		in yrs	(Kg)	(Cm)	Ave numbers of persons *	Income per month **
1	November 2003	7.60	20.46	118.71	(2 - 3)	(R250 – R3000) Ave income (R800)
2	September 2004	7.84	22.48	118.62	(2 -3)	(R250 – R3000) Ave income (R800)
	Total	7.73 +/- 0.60	21.93 +/- 4.82	118.69 +/- 7.23	(2 – 3)	(R250 – R999) Ave income (R800)

No. of people contributing to household income, **Average family wages per month in Rands is (R 250 – R3000) per month

Cu 1.26 – 2.99mg/l but the concentration in Sl_Zn 0.64±0.39mg/l was low when compared with standard at 0.5 -1.20mg/l. Sl_Mn was likewise low 0.11nmol/l when compared with the reference <40 nmol/L and showing a P value of 1 in both 95% upper and 75% lower confidence levels when compared with proportion meeting thresholds.

Comparing means against standards, there is a significant difference (P<0.01) between hair manganese and saliva manganese, also hair copper and saliva copper. Pearson correlation Coefficient showed a strong correlation between Sl_Mn and Hr_Mn (P<0.01), as shown in fig (1).

In other results levels of zinc, iron, copper and manganese were found in samples; water, cultivated vegetable and most commonly eaten foods, although these levels were high but within the range of reference values. Other factors like weight and height showed no significant difference within anthropometric data, although there was a significant difference in the socio-economic status of children but these did not influence the levels of these elements; zinc, iron, copper and manganese in both hair and saliva of learners investigated.

Discussion

This study has highlighted the possibility of using noninvasive method in investigating the presence of different elements in humans and can be compared with other researchers[1,22]. Hair provides more accurate and relatively permanent information on levels of exposure and the implication of the lack or excess of one trace element being able to interfere with the metabolic utilization of another element present as observed with excess copper lowering zinc levels and produces hair loss, insomnia, depression and schizophrenia and this can be found in different correlation factors found with and between parameters[11]. Same can be observed in saliva where more laboratories and clinic are relying on saliva for diagnosing and treatment of different diseases and due to the relative easy with which samples can be collected and analyzed, and confirm the relationship between salivary trace element concentrations and dietary intake as reported in literatures[9,23].

The cost-effectiveness of this method when compared to blood analysis in disadvantage communities has sparked our interest in the potential valuation of zinc, iron, copper and manganese in hair and saliva as an assessment index for trace elements[24.]

Table 3. Iron, zinc, copper, manganese in hair and saliva samples.

				Levels of trace elements hair and saliva samples and references			
Trace element	No. of Children	Trace Element index Conc.	Ref. standard Scale	% within standard range, below and above			Standard Deviation
*	N		**	Within (%)	Below (%)	Above (%)	
Hr_Fe in mg/kg	193	8.47	6 - 15	86	10	4	0.99
Hr_Zn in mg/kg	193	172.08	150 - 250	3	95	2	0.44
Hr_Cu in mg/kg	193	12.7	15 - 35	60	20	20	1.31
Hr_Mn in mg/kg	193	0.67	0.2 - 0.8	3	2	95	0.44

SI_Fe in mg/L	247	1.06	1.52 - 5.72	5	94	1	0.49
SI_Zn in mg/L	249	0.64	0.5 – 1.20	45	52	3	0.39
SI_Cu in mg/L	249	0.20	1.26 – 2.99	10	85	5	0.14
SI_Mn in nmol/L	249	0.11	<40	100	0	0	0.05

*Hr_ Pb = Hair lead, Hr_Cd = Hair cadmium, Sl_Pb = Saliva lead, Sl_Cd = Saliva cadmium, (**)Ref range.
Assaf and chung 1984, N = number of children involved in each analysis.

The result has show a strong correlation with manganese in both hair and saliva, with high percentages within reference which can indicates a disease free condition (manganism) resulting from lack or chronic manganese exposure, as characterized by different symptoms,[25] possibility of adverse effects of Mn accumulated *in utero* on 3-year-old children's psychomotor performances[26] which might be observe in later years.
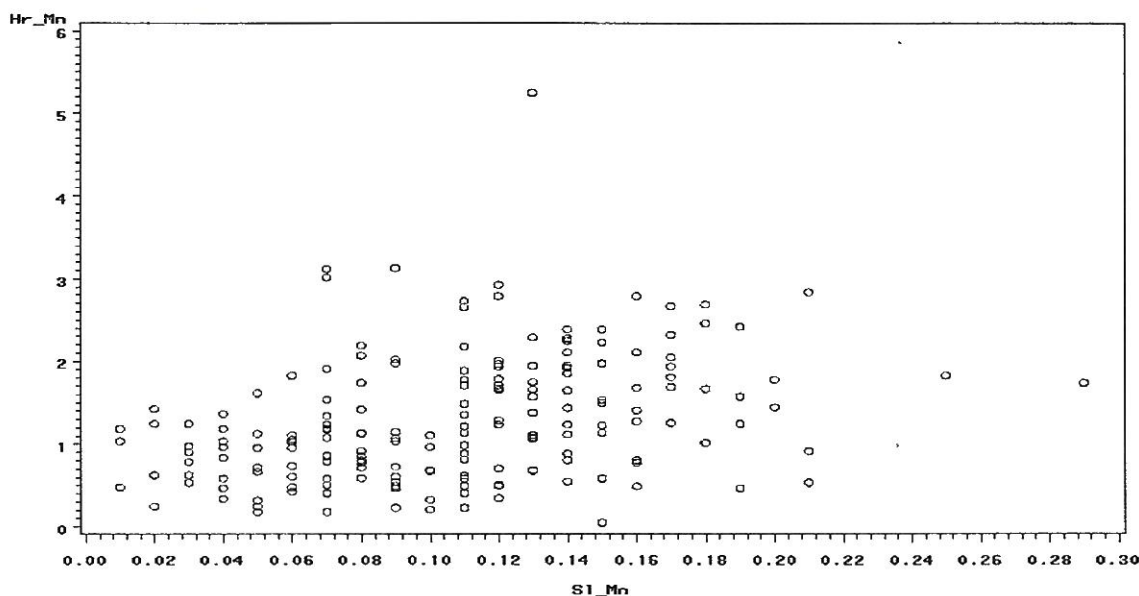

Figure 1: Graph of dispersion for hair Mn and saliva Mn showing strong correlation.

The addition of large amounts of zinc to a diet interferes with intestinal copper absorption that results in copper deficiency in spite of adequate copper intake. Hence, zinc is said to antagonize copper absorption. This is in compliance with the levels of these elements found in both food and cultivated vegetables which might have balanced the utilization in children samples. The potential Iron deficiency during childhood observed in developing countries although not observed in this survey has multiple consequences like neurochemistry disorder; alteration of dopamine receptors[27] and decreased monoamine oxidize activities[28]. Studies have linked iron deficiency with lead toxicity, and have provided evidence that these two conditions are related. [29]

This result provides a very significant study as the both indicators showed high level of iron and lead, complementing the growing evidence that high iron intake and iron-replete status may reduce lead absorption in children,[30] thus, the prevention of iron deficiency may represent a potential public health intervention for reducing lead exposure in humans,[31] and this can be

observed in levels of lead found in hair samples of children investigated.[32]

Studies like these will enable researchers pre-empt the possible outcome of subsequent findings but caution most be taken as concentration of an element in parameter might not indicate the same with others (saliva, blood and urine) and they might exhibit different concentrations even though the samples are collected at the same time.[32] The lack of standard procedure for sample collection, washing, treatment and analytical quality control protocol arouses considerable resistance towards the reliability of noninvasive techniques.[33]

The role of external factors responsible for varied levels of these elements found in children cannot be underestimated. Hence, the influence of the dietary intake is very significant (e.g. commonly eaten food, locally cultivated vegetables). The influence of other factors like weight, height and age showed no significant effect likewise the socioeconomic status did not play any significant role and can be attributed to the lifestyle of the people.

CONCLUSION
All over the world, trace element deficient has been a major issue of problem and critical evaluation of these elements will assist in diagnosing most food related illness, but not finding easier and less expensive technique will hinders this process, especially in rural communities were poverty is high. The use of noninvasive parameters as indices of trace element evaluation as shown in this study will ameliorates this problem. Although, this methodology needs further investigation and conclusions drawn on best approach, precautions, standards and references will enable it becomes workable process.

The reliability of trace elements in hair and saliva as indices of trace element status could be markedly enhanced when combined with other related indices measured simultaneously. Hence, the use of blood and other parameters should be encouraged. The interaction of trace elements in the body may have a dramatic impact on the utilization of other nutrients. Be it essential/non essential or micro/macro nutrients and help define the outcome of dietary improvement mechanism now adopted by various governments (dietary supplementation and food fortification programmes).

However, careful observation and diagnostic examination of cases with abnormal results will establish the presence or evidence of some form of diseases such as inflammation, infection and malignancy undermining the health status of the children, and this can be triggered by low nutrient intake resulting from poverty or poor nutrient intake. Trace element can be best evaluated through a simultaneous comparison of a variety of biochemical and physiological parameters of trace elements, Anorexia, short stature, and low level of hair nutrient.

Limitations
Despite the limitations of this study, such as self-reported subjective estimations of exposure, our observations suggest that environmental anthropogenic sources, especially environmental contamination, eating habits, acid interference and human error may contribute to increase in levels of these trace elements, it remains potentially sensitive fraction of the general population. More research is needed to objectively identify these sources and their associations with increased in some elements found in some samples and to assess the effect of their intake and subsequent child development taking into account the role of trace elements in metabolic activities in human body.

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