J. Basic. Appl. Sci. Res., 1(8)818-824, 2011

ISSN 2090-424X
Journal of Basic and Applied Scientific Research
www.textroad.com

Copper and Zinc Concentration in Hair of Healthy Children in Ceres District of South Africa

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ABSTRACT

Background: The aim of this study is to evaluate the presence of trace elements; copper and zinc in children using the non-invasive biomarker (hair). The effect of socioeconomic status on growth indexes and trace element utilization in children under investigation, also considered is the role of environmental factors.

Design: Copper and zinc status of first grade learners was assessed in N256 children age 7-9years, attending six primary schools in Ceres district of the Western Cape, South Africa. The children's copper and zinc status were evaluated over 2 academic years. The influence of socioeconomic status and anthropometric data and other factors like; zinc and copper in commonly consumed foods, drinkable water and locally cultivated vegetables were also investigated.

Results: There was a significant (P<0.01) presence of zinc and copper in the biomarker considered; noninvasive (hair). The following results were obtained (Hr_Zn 172.08±4.49mg/kg SD 62.45; Hr_Cu 21.03±0.79 SD 9.78. There was no significant correlation between Zn and Cu (P>0.01) in hair. Other factors considered showed considerable high levels of both elements in them, although significant variation in socioeconomic status but had no significant influence on the levels in children likewise their anthropometric data.

Conclusion: Comparing both trace elements, there was no significant lower mean level of both elements when compared with the standard means (P>0.01). Results show above 50% of both elements within standard reference values in all parameters investigated.

Summary: The study has identified the presence (copper and zinc) in children hair and has not been influenced by either the socioeconomic status or the Anthropometric data as observed in this survey.

The levels of this elements in other parameters such as; food, water and cultivated vegetable did not influenced this results, which indications the possibility of hair been used as a non-invasive biomarker.

KEY WORDS: Zinc, copper, hair, children and Ceres.

INTRODUCTION

Trace element evaluation in children and humans in general is a foremost important index of determining the state of health, immune status of children in a bit to combating increasing rates of food born diseases and infectious diseases, coupled with the ever increasing number of new diseases like HIV/AIDS, cancer, tuberculosis, fever, various kinds of flues and escalating allergies (Al-Numair, 2006, Dong, *et al* 1998; Labadarios, *et al* 2001). As man constantly look to nature for the nutrients required for healthy growth of children by using natural anti-microbial agents, which can assist the immune system and improve the health of children for a strong healthy life (Matthew, *et al* 2009 and Muzaffar, *et al* 2009).

There has been a huge interest in developing an alternative analytical method to monitor the presence and levels of trace elements in children apart from invasive (blood) especially in developing countries, as it will ameliorate most challenging issues that affects diagnostic and analysis of elements contamination such as; high cost, uneasy method of sample collection, and lack of a very reliable method and can be done through; hair, nails and saliva (Priya and Geetha, 2010; McLean *et al* 2009 and Taneja, *et al* 1998). Hair is a complex matrix, formed over a period of time, consisting of high levels of both organic and inorganic materials. Hair can provide a more permanent record of trace element associated with normal and abnormal metabolism as well as trace element assimilated from the environment (Hashem and Abed, 2007).

The advantage of the use of hair is that it can easily be collected, conventionally stored and treated, also it can be analyzed without any complex technique required (Dong *et al* 1998 and Khuder *et al* 2007). Hair is a noninvasive biomarker, capable of analyzing trace element, especially elements that can be associated with toxicity and those with a longer half life (Hashem and Abed, 2007). The use of other biomarkers such as blood have once been considered for its

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ethical reason but due to more and reliable analysis done on hair it is now considered the more accurate in elements evaluation in human body (Vishwanathan, *et al* 2002).

It is becoming increasingly clear that nutrition plays an important role in the general health of children and their ability to make satisfactory progress in school. Zn and Cu are essential in the growth and development of children (Liuzzi, *et al* 2001; Pfaffl and Windisch, 2003 and Olivares and Uauy, 1996). Information on nutrient status is of great importance to individuals, communities, as well as being of scientific interest (Albrink 1983; Brown *et al* 2002; Department of Health (1988) and Islam, *et al* 2003).

The use of multi-element hair analysis to assess an individual's nutritional health or predisposition to disease has been controversial, even denounced over the years due to lack of studies, which can correlate the low (Lurakis and Pitone, 1984) concentrations of essential elements in hair to deficiencies in tissue and body levels. It have been scientifically proven that trace element analysis in hair is useful for the evaluation of person's general state of nutrient and health (Hashem and Al-Othman, 2001), also it is valuable in detecting predisposition to disease and can assist Doctors in determining if a patient is suffering from trace element deficiency or trace element in-balance or heavy element pollutants in the body which may be responsible for a particular illness (Labadarios, *et al* 2001 and Priya and Geetha, 2010).

Hair zinc values are a good indicator of nutritional status (Weber, *et al* 1990) and supplementation with zinc (Cavan, *et al* 1993 and Leung, *et al* 1999) can be enhanced in hair zinc deficiency. Alternatively, the effect of a toxic trace element may be ameliorated by another protective trace element (Cavan, *et al* 1993), this can be observe when large amounts of zinc in a diet interferes with intestinal copper absorption and results in copper deficiency in spite of the adequate copper intake, hence zinc is said to antagonize copper absorption. Copper deficiency in turn, is also known to provoke iron deficiency and anemia (Cavan, *et al* 1993). Zinc, a nutritionally essential trace element, it can influences both the absorption and the toxicity of lead. The interaction between environmental lead and environmental zinc levels and blood lead concentrations has suggested that zinc may influence the association between soil, dust lead and corresponding blood lead levels (Noonan, *et al* 2003 and Taneja, *et al* 1998).

METHODOLOGY

Study design

This study is aimed at assessing the levels of zinc and copper in hair (noninvasive). The techniques used (Aqua regia) is widely used for the determination of trace elements in matrices, especially biological materials. Both elements were analyzed in all grade one learners in primary schools in Ceres district of the Western Cape, South Africa.
Prior to data collection, parents of the children under investigation were asked through questioner, if they had any direct contact with objects that might increase their venerability to trace elements contamination. Where the answer was a yes, the samples collected were discarded and not included in the analyses.

Study population and study sample

The study population consisted of 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. 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 copper and zinc in hair samples by conventional *Aqua Regia* digestion method

The determination of copper and zinc in hair, using the conventional *aqua regia* digestion procedure, consists of dissolving of samples in a 3:1 mixture of HCl and HNO3 and digested on a hotplate for 3hrs (Nieuwenhuize, *et al* 1991). A photometric method was used in analyzing the digested samples using an atomic absorption spectrophotometer (AAS) (Unicam AAS Type solar) (Smith, *et al* 1979 and Vercoutere, *et al* 1975).
Spectrophotometry About 5mL of digested samples were analyzed using the AAS at a wavelength most suitable to the particular element being 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 caused by acid interference, common with the *aqua regia* method. In order to obtain reproducible results, it was important that we maintained constant optimal aspiration and furnace conditions. All machine readings were repeated twice.

HAIR

Approximately 0.5g of hair, 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 ethanol after each hair collection.
Hair samples were washed with non-ionic detergent and rinsed with distilled water, oven dried for 4 days at 60-70oC and stored in an airtight plastic bag. 0.25g well-mixed dried hair was weighed into a beaker and digested in 12mL of aqua regia (1/3 HNO3 and 3/4HCl) acid, heated in a heated Gerhardt (Trace metal digestion units, DIN 38414) 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 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 doubly deionised distilled (DDW) water (Moore and Chapman, 1986). This solution was stored in a plastic container until analysis using AAS was performed. The levels of copper and zinc in hair were determined using the AAS.

Table 1: Characteristics of study sample

Years	Total No. of learners in six schools	Number of learners selected for inclusion.	Number of learners included in survey (consent received + present on day of data collection	Proportion of selected learners who participated	Response rate (%) of Girls/Boys
November. 2003	544	200	115	60.0	43/57
September 2004	688	226	150	64.5	54/46
2003/04	1232	426	265	62.3	

Table 2: Socio-economic characteristics of learners

		Age of participants (years)	Weight (kg)	Height (cm)	Family members contributing to household income	Family average wage
Phase One		Avgas in yrs	(Kg)	(Cm)	Income *	
1	November 2003	7.60	20.46	118.71	4.82	2.19
2	September 2004	7.84	22.48	118.62	4.12	1.84
1 and 2	2003/04	7.73 **SD** 0.60	21.93 **SD** 4.82	118.69 **SD** 7.23	4.73 **SD** 1.28	2.73 **SD** 1.24

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 voluntary 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 **(SAS Institute Inc., 1999)**. The results are presented as mean, standard deviation and Pearson Correlation Coefficient between zinc and copper in hair. The P-values >0.01 were considered statistically not significant.

RESULTS

Table 3: Levels of Zinc and copper in Hair samples and references

Levels of trace elements in samples and references.					
S/N	**Trace elements**	**Levels of element**	**Reference standards**	**Reference standards**	**Reference standards**
		in subj. (mean)	without scales	with scales	Values (mean)
	*				
1	Hr_Zn	172.08	150-250	150-250mg/kg	200
2	Hr_Cu	21.03	15-35	15-35mg/kg	25

* Hr_Zn (hair zinc), Hr_Cu (hair copper)

Tables 1 and 2 gives the results of mean values obtained in the anthropometric data of healthy children, grouped according to percentage participation and ratio of boys and girls. The ration of age bracket, weight and height, also obtained include the socioeconomic status of parents represented in the family member contributing to house hold income and family average wages.

All these data in table 1 and 2 gives illustrates to the role of anthropometrics information and socio-economic status of the children and was discovered to have no significant influence on the levels of this essential trace elements (copper and zinc) availability in the children. Table 3, shows the frequency distribution, mean, median, concentration and standard references are shown in table 3. (Hr_Zn 172.08±4.49mg/kg SD 62.45; Hr_Cu 21.03±0.79 SD 9.78). The mean (±S.E.) ratio of both copper and zinc are with the standard book reference. Zinc and copper concentration in samples showed no significant correlation between standards and hair means (P>0.05) representations.
Comparing standards there was a significant difference (P<0.01) between Hr_Zn and Hr_Cu. Anthropometric data show no significant difference in age, gender, weight and height, although there was a significant variation in socioeconomic status but had no significant effect on the levels of both elements in children. Different levels of both elements were found in food, water and cultivated vegetables analyzed, although all were within standard book reference.

DISCUSSION

The mean hair concentration (150-250mg/kg for Zn and 15-35mg/kg for Cu) obtained in this study is within the book reference values for zinc and copper as reported in (Ekhard, *et al* 1996). The anthropometric data and socioeconomic status did not influence the concentration, contrary to what was previously observed by Veith JW in his work (Veith, 1988) and (Waslien and Rehwoldt, 1990) respectively. However similar result was observed in serum (Diaz Romero, *et al* 2002 and Al-Numair, 2006). In children under investigation, there was non significant difference between elements (Zn and Cu), but a significant difference within hair $p<0.05$ samples, indicating various changes in the content of these elements in samples taken from these children.

The mean concentration of zinc is relative higher than the copper in hair but are within the reference and might have been affected by age bracket or the gender of the children been investigated, this is in compliance with the finding of Taneja SK, suggesting that young healthy NIDDM descendants possess a high-Zn and low-Cu reserves in their hair and the observed concept appears to be associated with Cu-Zn antagonism (Taneja, *et al* 1998). It is important to note that this children showed no subnormal provision of these trace elements and no clinical symptoms contrary to (Kon'IIa, *et al* 2001), which indicated that in investigation carried on Moscow children showed subnormal trace elements provision and is not accompanied by clinical symptoms of their deficiency.

Bales, *et al* 1990 in their contributing to the use of noninvasive biomarker has indicated that copper in saliva was positively associated with concentrations in plasma but negatively related to concentrations in hair, also (Dastych, *et al* 2008), in their investigation has indentified that various changes in the content of various elements in hair samples taken from this children has not been by accident but might be due to a shift in our knowledge in the speciation of various forms of trace elements in the different organs in the human body. Arnaud, *et al* 1993, in 'Nutritional biological markers of deficiencies of zinc, copper and selenium' has indicated that despite the numerous indices used in trace elements analysis, none of them provide the same and satisfactory assessment of the nutritional status for those trace elements buttressing the finding of (Dastych, *at al* 2008) and the use of multiply biomarker if accurate result is to be achieved.
The result of this survey is a contrast to the popular view of South African socioeconomic status data base, which states that population of people in the country that are poor is estimated at ninety five percent and are African and 75% of the poor live in the rural area, similar to what is obtained in Ceres (http//www.statessa.gov.za/Statistical_release/Statistical_releases.htm) and could result in nutrient deficiency due to reduce nutrient intake but contrary to the results it has been observed that levels of Zn and Cu essential trace elements are within book reference and all the children taking part in the survey are of good health and is in agreement with other findings (Shakur, *et al* 2004 and Krebs and Hambidge, 1986).

This results has show levels of Zn in both biomarker is an indication of lack of deficiency as against the possibly evidence of widespread Zn deficiencies in developing countries (David, 2004 and Bhutta, *et al* 2000). This was observed in a meta-analysis of supplementation studies carried out in children below 13yrs which showed that there was a high significant positive impact of Zn on the increase in weight of children (Brown *et al* 1998).
Researchers have also recognized that trace elements exposure and mineral deficiencies shown in the hair of children, is the diary of what is going on in their body and suggesting that the metabolic pathways of zinc and copper are as similar as those found with other diagnostic procedures (Lescure, *et al* 2002), this can be associated with this results.

The different levels of zinc and copper found in other parameters analyzed (food, water and cultivated vegetables) is an indication that hair analysis provides more permanent record of trace elements associated with normal and abnormal metabolism as well as trace elements assimilated from the environment (Labadarios, *et al* 2001). The reliability of these parameters (noninvasive) is that, hair can easily be collected, conventionally stored and treated also without any complex technique required (Ogboko, *et al* 2009).

CONCLUSION

It has been well established that presence of excess essential trace elements produces adverse health effects on human beings. For virtually all chemicals, adverse health effects are noted at sufficiently high total exposures levels

likewise in reduced conditions. The primary purpose of the survey is to evaluate the levels of these essential trace elements; copper and zinc in hair of these children.

This survey has identified the presence of different levels of trace elements (Zn and Cu) in these children and it is the first evaluation of trace elements analysis carried out in healthy children of Ceres district, also due to elimination of all critically infected children and all value that are outrageously high or low. Likewise the effect of socio-economic status, the trace elements levels in children and this has not affected both. Although few children are within the lowest economic scale but still show presence of these trace elements availability and this could be due to similarities in diet, eating habit, culture and trace element absorption and it has also shown that there are high concentration of zinc and lower concentration of copper compared to book reference obtained from similar studies.

With significant levels of Cu and Zn found hair of children investigated there is a tendency that this finding might have been influenced by the homogeneity of anthropometric data and commonly eaten diet, as well as the possibility of homogeneity of the single area used in the investigation (**Ceres**), hence more broad based survey is needed across other districts in South Africa.

The non interference of major factors like anthropometric data and socioeconomic status is clear indication that the environmental factors most have played some part, reasons been that other parameters like food, water and cultivated vegetable showed standard levels of Zn and Cu in them. This finding has also shown that the associations between hair and other parameter analyses is not parallel and those demographic variables have not been found; fewer studies have shown a correlation between essential elements in hair of animals and the same elements in organs. It is possible to measure trace elements in hair to satisfy the skeptical chemist and for diagnostic purposes.

Although the conclusion is speculative, the findings may offer an avenue for further research. It is worth pointing out, that while testing of this kind may be informative for research, the current state of knowledge on the practical implications of clinical management is limited.

LIMITATIONS

The findings in this report are subject to some limitations, which include the wide range of results obtained from the use of the *aqua regia* method known to be susceptible to acid interference. Although parents of children were asked to give information on the use of creams, shampoo and other chemicals on the children's hair, there are possibilities of foreign substance interference. This report provides a snapshot on how information on element contaminations can be collected from children within a short time and is one way of monitoring the state or progress of children affected by contamination. Finally, there is an indication that the presence of these elements might have resulted from prolonged environmental exposure to these elements.

ACKNOWLEDGEMENT

I acknowledge the following person for their immense contribution towards the successful completion of this paper; Rina Swart, David Fisher, Lincoln Raitt, Lilburne Cyster, staff and students of Human ecology and Dietetic department.

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