

Selenium in Saliva and Hair of School Going Children in Ceres District of the Western Cape South Africa

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ABSTRACT

The concentration of essential trace element selenium was evaluated in saliva and hair of school going children in a diverse socioeconomic community of Ceres district in the Western Cape. N265 grade one learners were involved in this survey. The analytical results of selenium levels of children (age 6–9) were $0.85 \pm 0.8 \mu\text{g/kg}$ for hair and $0.42 \pm 0.7 \mu\text{g/l}$ with no significant difference between genders ($p = 0.659$). There is a strong correlation ($P < 0.01$) between selenium in hair and saliva. The mean concentration between hair and saliva is significantly lower ($P < 0.01$) than the standard mean values. Similar results were observed in other parameters investigated which include; food, water and locally cultivated vegetable. Although low in concentration but are within book reference and had not influenced the selenium levels in both hair and saliva. Other factors like anthropometrics data; age, weight, height, gender and socio-economic status, were also invested with socio-economic status showing significant difference but has no major effect on the results and this can be attributed to the small age bracket, nutritional intake of other nutrients and some time genetic influences do play a role in nutrient bioavailability. This low selenium levels observed in this study had not suggested that the children are endangered by an extremely low selenium status.

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 for the practical implications of clinical management is limited.

KEYWORDS: Hair, saliva, selenium, children and Ceres.

INTRODUCTION

Selenium (Se) is an essential trace element, a micronutrient that exerts its biological functions through the biosynthesis of different numbers of selenocysteine-containing proteins (Kryukov GV 2003). Nutritional deficiency of selenium decreases the expression of the selenoproteins and thereby impairs selenium's biological functions. The role and requirement of selenium in human health has been well documented over the years, this was complemented with the knowledge of selenium having well over 25 different proteins namely selenoproteins (Savarino L *et al* 2001).

The trace element selenium is an essential component of several metabolically important enzymes (selenoprotein) which include the antioxidant glutathione peroxidases and thioredoxin reductases (Rayman MP 2000), isolated from human erythrocytes and type 11 thyroxine-5-diiodinase (Schamberger RJ 1986, Stadtman TC 1990). The function of most selenoproteins is currently unknown; however, selenoprotein plays a role in antioxidant defenses as a component of glutathione peroxidases (GPx), thioredoxin reductases (TrxR), and thyroid hormone diiodinases (DIO). These selenoprotein characters are involved in redox regulation of intracellular signalling redox homeostasis and thyroid hormone metabolism (Lutz Schomburg *et al* 2009).

Selenium is essential for the effective operation of many aspects of the immune system both in animal and man. Immunity and the immune system are a very complex collection of processes that act together to protect organisms against attacks by pathogens and malignancy (John RA *et al* 2003). Most immune functions involve inflammatory mechanisms that when uncontrolled may be implicated in the pathogenesis of conditions such as coronary heart disease, cancer, immunity and rheumatoid arthritis, are influenced by selenium presence or absence (Lescure A *et al* 2002, Kryukov GV and Gladyshev VN 2002). Recent studies has identified selenoproteins in the prevention of some forms of cancer, a number of clinical trials has been carried out and more are underway or being planned to examine the effects of selenium on cancer incidence.

Selenium is an active part of glutathione peroxidase (GSH-Px), an antioxidant enzyme (Rotruck JT 1973). It has been shown to respond to oxidative stress when taken as a supplement, through the modulation of cellular reaction inducing a quicker restoration of the endogenous antioxidative defense

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system against the production of reactive oxygen species (Jozanov-Stankov O 1998). Nutritional deficiency of selenium decreases the expression of the selenoproteins and thereby impairs selenium's biological functions. This could result in a decline in tissue selenium-dependent glutathione peroxidase activity (Burk RF 1983). Oxidation low density lipoprotein cholesterol could induce endothelial damage and thus facilitate the atherogenic process by allowing the entry of elements from the blood and allowing the adherence of platelets (Steinberg 1991). Some scientists have proposed that glutathione peroxidase protects endothelial cells from the effects of oxidized low density lipoprotein cholesterol (Meister A 1992).

The concentration of selenium (Se) in human organism varies widely between geographical areas depending on its content in soil and plants, dietary Se intake, bioavailability and retention, mineral interactions and other factors that determines the availability of selenium in humans, also concentrations of selenium varies within organs of the body (Case AJ, *et al* 2010, Smith LA, *et al* 2010 and Ovaskainen M-L *et al* 1993).

Dietary selenium intake reduction can reduce the intake of essential trace elements, which play indispensable roles in various physiological and active functioning of the immune system (Jackson MJ, *et al* 2003). The trace elements zinc, copper, and selenium are often deficient after prolonged enteral nutrition (M Kumode 2003). Dietary selenium is essential for an optimum immune response, although the mechanisms of this requirement are not always fully understood. Selenium influences both the innate, "nonadaptive" and the acquired, "adaptive" immune system (Beckett GJ *et al* 2003), the innate immune system include barrier to infection and nonspecific effectors cells such as macrophages. Both T and B-lymphocytes form the major effectors cells of the acquired system that mature with exposure to immune challenges (Turner RJ and JM Finch 1991, Pfeifer DM *et al* 2001, McKenzie RC *et al* 2001, and McKenzie RC *et al* 2002).

The existence of selenium in both organic and inorganic form could account for the existence in human metabolism, a kind of mechanisms that could account for the inverse relationship between cadmium and whole blood selenium, where high whole-blood selenium concentration, is a consequence of high selenium intake in a mother, and may decrease the transportation of cadmium into placenta by changing cadmium distribution (Kantola *et al* 2000, Satoh *et al* 1981). Selenium (found in seafood, liver and kidney and in small amounts in other products like meats, grains and seeds) helps maintain selenium balance, healthy heart, eyes, liver, skin and hair.

Selenium has been identified in different organs of the human body such as; hair, nails, blood, urine and saliva, although the concentration varies significantly, the importance can some times be undermined. In this study the role of hair and saliva is been considered. Hair is a unique biological specimen that shows the concentration or profiles of elements intake over a long frame of time without showing daily transient changes (RJ Shamberger 2002, DA Bass *et al* 2001). Hair is easy to obtain, thus the usefulness of investigating the selenium status in these children is very important. Selenium levels in hair have been shown to positively correlate with those in the plasma, kidney, liver, and lung (Shamberger RJ 2002) and (Hac E *et al* 2002). Hair selenium values are very low in selenium-deficient children, and the concentration can be increased in a dose-dependent manner using selenium supplements (Shamberger RJ 2002).

Human body fluids such as saliva is presently been used in the diagnosis of different diseases, as such remain useful in the analyses of selenium in human. Saliva is a complex mixture of proteins. The higher accuracy of a saliva test is a fact to most recent levels of trace elements analyzed and can be observed in the study done by David Gutierrez (2009), also saliva proteins are much easier to detect in saliva than the proteins in blood (David Gutierrez 2009). There has been a significant positive correlation between GSH-Px and Se contents and also between protein and Se contents expressed per volume for human saliva, erythrocyte and the whole human fluids as investigated in (Hojo Y 1987).

METHODOLOGY

Study design

This study aimed to assess the level of selenium in hair and saliva. This technique is widely used for the determination of trace elements in matrices, especially biological materials (Benson Ogboko 2011). Selenium was analyzed 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 questionnaire whether special shampoo, relaxer, or cream were used on their hairs, and also if they had any direct contact with objects that might increase their vulnerability to any element 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.

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
		7.73	21.93	118.69	4.73	2.73
1 & 2	2003/04	SD 0.60	SD 4.82	SD 7.23	SD 1.28	SD 1.24

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 selenium in hair and saliva samples

The determination of trace elements (selenium) in hair and saliva, using the conventional *aqua regia* digestion procedure, consist of dissolving of samples in a 3:1 mixture of HCl and HNO₃ and digested in a hotplate for about 3hrs (Nieuwenhuize J *et al* 1991). A photometric method was used in analyzing the digested samples using atomic absorption spectrophotometer (AAS) (Unicam AAS Type solar) (Vercoetere *et al* 1995, Abramovitch RA *et al.*, 2003 and Benson Ogboko, 2011).

Spectrophotometry: About 5mL of digested samples were analyzed using the AAS 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 caused by acid interference, common with *aqua regia* method. In order to obtain reproductive results, it was important that we maintain constant optimal aspiration and furnace condition. 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 surgical spirits after each hair collection.

Hair samples were 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. 0.25g of 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 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 and 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 double deionized distilled water (DDW) (Moore PD, SB Chapman 1989 and Ogboko *et al* 2009). This solution was stored in a plastic container until analysis using AAS was performed.

SALIVA: A minimum volume of 5.0mL mixed saliva was collected from the learners into a detergent washed polypropylene vial by direct 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 (HNO₃) was added. This solution was then filtered with Whatman no. 42 filter paper into a volumetric flask and diluted to a final volume of 100mL with DDW (Moor PD, SB Chapman 1989 and Ogboko *et al* 2009). The 100mL solution was then stored in a plastic container until analysis with an AAS for selenium 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 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, 1999). The results are presented as mean, standard deviation, and Pearson Correlation Coefficient between selenium in hair and saliva. The P-values <0.01 were considered statistically significant.

RESULTS

Tabel 3: Levels of trace element selenium in samples and references

S/N	Trace elements	Levels of element	Reference standards	Reference standards	**
		in subj. (mean)	without scales	with scales	Reference standard
					Values (mean)
1	Hr_Se	0.85	0.5 - 2.0	0.5 - 2.0ug/kg	1.25
2	Sl_Se	0.42	0.75 – 2.40	0.75 – 2.40ug/l	1.57

Hr_Se = Hair selenium, Sl_Se = Saliva selenium, (**)Ref range. Malvy DJ *et al* 1997, N = number of children involved in each analysis.

The MEANS Procedure

Variable	N	Mean	Std Dev	Minimum	Maximum
sn	265	132.5849057	77.0005003	1.0000000	265.0000000
Trials	265	1.5547170	0.4979374	1.0000000	2.0000000
CODE	265	3112.49	1671.73	1001.00	6015.00
Sl_Se	242	0.4183471	0.7223697	0.0200000	5.0600000
Hr_Se	190	0.8451053	0.7513295	0.0500000	4.6200000

Pearson Correlation Coefficients

Prob > |r| under H0: Rho=0

Number of Observations

Hr_Se

Sl_Se 0.24772
 0.0008
 180

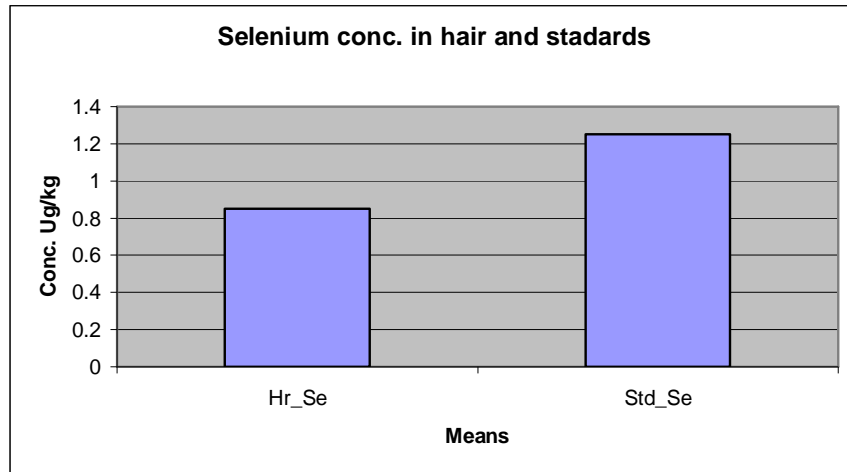


Figure 1. Hair selenium levels in children against standard reference.
Mean hair selenium levels in $\mu\text{g/kg}$ and Standard in $\mu\text{g/k}$

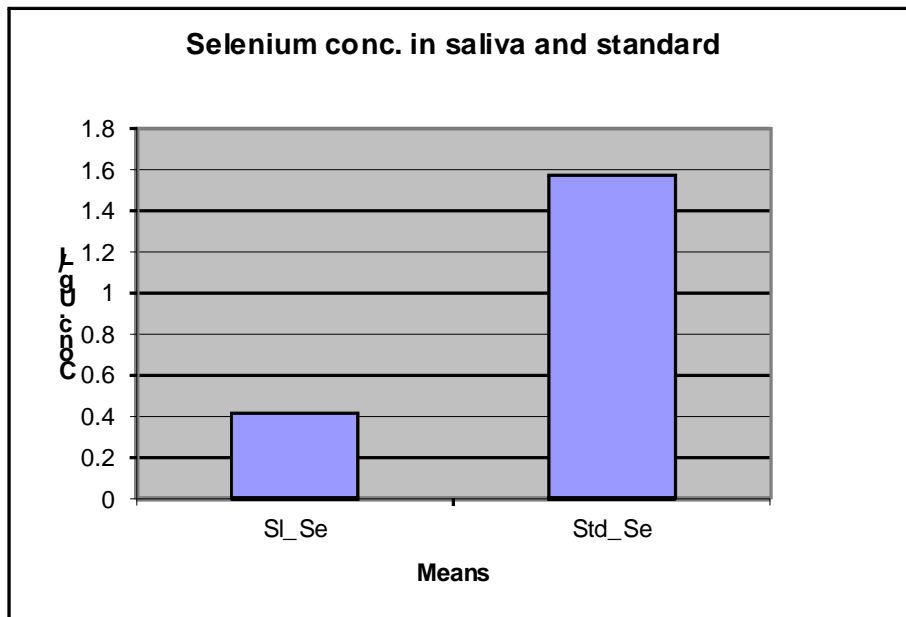


Figure 1. Saliva selenium levels in children against standard reference.
Mean saliva selenium levels in $\mu\text{g/L}$ and Standard in $\mu\text{g/L}$

The following mean concentrations were observed (Hr_Se $0.85 \pm 0.05 \mu\text{g/kg}$ SD 0.75; Sl_Se $0.42 \pm 0.05 \mu\text{g/l}$ SD 0.72 0.42). In comparison the mean concentrations with the standard means of $1.25 \mu\text{g/kg}$ for hair, and $1.57 \mu\text{g/l}$ for saliva, the selenium (Se) concentrations in the above learners were significantly lower ($p < 0.01$) than the standard mean. Individual selenium levels showed a deviated distribution in comparison with the book references, and statistical significance showing a positive correlation between both samples and means.

The statistical analysis carried out using both SAS (Pearson correlation Coefficient) and the SPSS 13 version (Pearson correlation Coefficient) in comparing correlation mean factors between saliva and hair showed a significant correlation ($P < 0.01$). In comparing means, there was a significant difference in both means (sample means and standard means). Hair selenium mean is significantly lower than the standard mean (StD mean $1.25 \mu\text{g/kg}$ and $0.85 \mu\text{g/kg}$ in hair respectively) and (StD mean $1.57 \mu\text{g/l}$ and $0.42 \mu\text{g/l}$ in saliva respectively).

The result basically showed a significant decrease in selenium levels in both hair and saliva. Other factors like anthropometrics data; age, weight, height, gender and socio-economic status, were also investigated, the result show similar trend; no significant difference in the anthropometric data,

although there is a significant difference in socio-economic status but has not influence the levels of selenium both in saliva and hair. All samples analyzed contained selenium in varied amount depending on the item (food, water, vegetables) analyzed signifying the viability of the procedure but could not ascertain the exert amount required by this children. This data did not affect the levels of trace element (selenium) in children significantly.

DISCUSSION

Selenium can be expressed as the concentration of Se in hair and saliva as it reflects recent dietary intake, and can as well represent, short and longer-term indicator of selenium status as often expressed as the concentration of Se in serum (plasma) (Longnecker *et al* 1996). The use of hair and saliva as a biomarker for assessment of trace element status in human is gradually been accepted for most biological and medical research (Venclíková Z, *et al* 2011), although this study has confirmed it usefulness it need more acceptance as serum is considered the major biomarker in Sub-Sahara Africa and in the developing world, solely due to ethical reasons. This technique has identified different levels of selenium in food, water and vegetables as shown in most biomarkers.

The use of hair has a potential advantage in examining exposure to toxic minerals and the nutritional status of essential minerals because hair reflects the status of trace elements over a long time frame and is not transiently disturbed by recent factors that may affect it, making hair an important biomarker. Nevertheless, the interpretation of the results of hair measurements is sometimes difficult because the systemic distribution of selenium is not fully understood (Richelle M, *et al* 2006). It is likely that other absorbed trace elements heterogeneously affect the distribution of selenium among organs, which may cause discrepancies between the concentrations in hair and those in the internal organs of children.

Nonetheless, when there are significant changes, such as high or low levels of trace elements or malnutrition in various pathological conditions, the systemic changes in most elements like selenium might be reflected in different organs such as; hair, saliva, nails, blood and urine (Shamberger RJ 2002). There has been a correlation between selenium levels in hair analysis and that of selenium levels in blood and urine and in some cases saliva (Valentine JL, *et al* 1978), hence the need for research of this nature to evaluate the presence of this element in children is essential.

One of the factors that affects selenium composition in human is the dietary intake and this can only be made available in food consumed and this can vary widely depending on the soil content in which the foods are grown. The bioavailability of selenium in food plays a significant role in the health status of this children since their body, can not synthesis selenium, it must be made available through ingestion. Selenium is very significant in ones disposition to infection. Epidemiological studies has suggested that low intake of Selenium might predispose one to increased incidence of cardiovascular disorder (Salonen JT *et al* 1982, Kohrle J *et al* 2000).

Selenium deficiency is responsible for the prevalence and severity of iodine deficiency disorders a well-known global nutritional problem, most prevalent in developing countries (Ceres). Iodine deficiency in children impairs neuromotor and intellectual development disorder in later years (Napolitano G *et al* 1996). Selenium is also required in thyroid metabolism in children, converting inactive thyroid hormone into active thyroid hormone (Zeder C, Hurrel RF, 200). It has been shown that Se deficiency partially blunts thyroid response to iodine supplementation in goitrous children that are deficient in both selenium and iodine (Raines DA *et al* 1999). Some research has indicated a geographical link between regions of selenium-deficient soils and peak incidences of HIV/AIDS infection. For example, much of sub-Saharan Africa is low in selenium (Patrick L 1999), however this need to be further investigated,

Also the low level of selenium observed in the children under investigation could be attributed to various factors; like; dietary intake, eating habit, genetic make up and environmental contaminations, similar findings relate smoking to reduced selenium levels in human tissue (Ellingsen *et al* 1997), and can be linked to hair and saliva, which possibly explained the suppressant effect of cadmium in cigarettes on selenium levels in human tissue. Significantly lower blood selenium levels have been noted among smokers (Bates *et al* 2002b), and some of the learners parents are smokers or may have been polluted by partial smoke inhalation, and low selenium in diets; as observed in the commonly consumed food stuff analyzed showed a considerable low levels of selenium in them, also environmental selenium presence may have contributed.

The low levels of selenium in this study do not suggest that the learners are endangered by an extremely low selenium status, and they have not been accompanied by clinical symptoms of their deficiency (Kon' Iia *et al* 2001). There is also increased evidence of selenium deficiency coursing several serious short- and long-term medical implications, including impaired immune response, or

even cancer. An experimental study has shown that an increase in selenium level can be associated with decreased in cancer mortality and reduction in some types of cancers (Gupta S *et al* 1994). The mean Se level for healthy children (age 6–9) observed in this study was $0.85 \pm 0.8 \mu\text{g/kg}$ for hair and $0.42 \pm 0.7 \mu\text{g/l}$ with no significant difference between genders ($p = 0.659$). There is a significant low selenium levels when compared with book reference.

CONCLUSION

In conclusion, this survey has identified levels of selenium in both hair and saliva, although a lower concentration which could be as a result of other factors but this study has not suggest that the children are endangered by an extremely low selenium status as stated in the discussion. Other factors like anthropometrics data; age, weight, height, gender and socio-economic status, have not significantly influence the levels of selenium in both saliva and hair, this might be an indication of small age bracket, nutritional intake of other nutrients and some time genetic influences do play a role in nutrient bioavailability.

This study needs a long term monitoring of selenium status of the general population, judging from the nutritional benefits of selenium in the biochemical functioning of the human body. However, the relationships between the selenium in saliva and hair in deficient conditions and the threshold values indicating the need for alternative trials and should include the use of invasive (blood) method of analysis to individual and should apply to individual cases, as to elucidate the finding of low selenium in school going children in Ceres.

The concentration of selenium (Se) in humans varies widely between geographical areas depending on its content in soil and plants, dietary Se intake, bioavailability and retention, mineral interactions and other factors, hence the low levels of selenium in both hair and saliva could be attributed to geographical factor rather than socio-economic or anthropometric data. The significant low level of selenium found in both hair and saliva is similar to other parameters analyzed and can be attributed to geographical location of Ceres. In view of the relative easy with which selenium analysis can be done in hair and saliva more studies will help ascertain the accuracy of this technique, since no similar survey has been done in the area.

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 for the practical implications of clinical management is limited.

The reliability of trace element concentrations 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. In a situation where one element is not directly responsible for the cause of any particular illness, it is possible that it may still have a role in the cause of related disorders. The interactions of trace elements in the body may have a dramatic impact on the utilization of other nutrients. Be it essential/non essential or macro/micro nutrients.

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 trace elements can be collected from children within a short time and is one way of monitoring the state or progress of children affected by contamination or deficiencies. Finally, there is an indication that the presence of these element might have resulted from prolonged environmental exposure to these elements.

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