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Cadmium and Lead Concentration in Saliva of Children in Ceres District of South Africa

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ABSTRACT

Background: This study is to evaluate the presence of heavy elements (cadmium and lead) in children using the non-invasive biomarker (saliva). The possible source of these elements in children; food, water and cultivated vegetable were investigation,

Design: cadmium and lead status of first grade learners is assessed in N256 children age 7-9years, attending six primary schools in Ceres district of the Western Cape, South Africa. The grade one learner's heavy element status is evaluated over 2 academic years. The influence of socioeconomic status and anthropometric data from growth indexes is also investigated.

Results: There are high levels of lead in saliva (1.07 ±1.31µg/L), when compared with the standard values of <1.0µg/L. The level for cadmium in saliva (0.67 ±0.48µmol/L) is also high with 56% above average with the mean within the tolerable standard limits in the human body (< 0.55µg/L).

There is a significant (P<0.01) presence of cadmium and lead in other parameters investigated; food, water and cultivated vegetable, although high but not significant to course any health issue. However the influence of socioeconomic factor and growth indexes did not influence the presence of these elements

Conclusion: This study has shown the presence of these heavy elements (cadmium and lead) in children saliva, an indication of some form of contamination and the ability to evaluate the presence of these elements in saliva. It can be considered a useful biomarker in the search for non-invasive biomarker that is conformable to the norms of a rural environment like Ceres. This survey is a base line study; more similar studies will be needed to validate this finding.

KEY WORDS: Cadmium, lead, saliva, children and Ceres.

INTRODUCTION

Evaluation of trace elements in biological specimens (saliva) in children are scarce for non-essential elements (cadmium and lead), especially in Sub Sahara Africa (Torra M, *et al* 2002). The need for baseline analysis of these elements in children and in the population in general is very important, children experience most active period of their life; rapid growth, high energy and nutrient intake during which food habit and other life-style factors are very prevalent (Van Assche FJ, 1998 and Muhammad Muzaffar A, *et al* 2009). Hence the needs for cost effective and easy biomarker for trace elements analysis.

Increased agricultural activities and industrialization has resulted in high levels of heavy element contamination in urban and rural areas (A.R. Hashem and K.F. Abed Aluminum, 2007). It has prompted the need to make trace element testing a priority especially in children, as well as the role of heavy elements in creating adverse health effect, in the utilization of essential nutrients. Testing is important to the individual body so as to diagnose more accurately any deficiency diseases or overdose of any element and it reaction to other elements as to advice on an effective therapy or treatment. The most common heavy elements that basically affect children are lead and cadmium (National Academy of Sciences/National Research Council, 1996; Needleman H, 1993; Dixin wang, *et al* 1995 and Thomas VM, 19950). Saliva an oral fluid consists of enzymes, nutrients and antimicrobial compounds, and can be an excellent way of detecting heavy elements contamination in humans (Stephen George, *et al* 2002 and Muehlenbruch B, 1982).

Heavy elements like lead (Pb) and cadmium (Cd) in the human body normally comprise of less than 0.01% of body weight (WHO Technical Report Series No 940, 2007). Most heavy elements are usually absorbed into the human body either by ingestion, inhalation, or absorption through skin contact (Storelli MM, *et al* 2010). Information on the levels of many trace elements in biological tissues (saliva) is difficult to obtain and sometimes inaccurate especially in nonessential elements and acceptable tolerable limits in children are lacking (Agency for Toxic Substances and Disease Registry (ATSDR), 1997 and Watanabe T, *et al* 2002).

Cadmium can cause gastrointestinal disturbances such as nausea, abdominal cramps and diarrhea, stomach irritation, vomiting, salivation, convulsions, shock, kidney failure, and sometimes death. Short-term health effects include

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flu-like symptoms, such as chills, headache, aching and/or fever (Agency for Toxic Substances and Disease Registry, 1989; Kaneta M, *et al* 1980 and Abdulaziz A. Al-Khedhairy, *et al* 2001). Death is usually due to excessive fluid loss from vomiting and diarrhea (World Health Organization (WHO), 1992 and George Ware and David M Whitacre, 2007). Poisoning by inhalation may lead to respiratory manifestations such as severe bronchial and pulmonary irritation, sub acute pneumonitis, lung emphysema, and in the most severe situations, death from pulmonary edema may occur (Lauwerys RR, 1986). Cadmium interferes with metabolism of copper, responsible for hypertension and related heart diseases. Lead is highly toxic with an average tolerant rate in body at 1- 2mg (John D, *et al* 1984).

The harmful effect of lead and cadmium in child development will always occur without clinical symptoms and might not present any abnormality during routine test (Cheng Y, *et al* 2001). Factors that predispose lead toxicity include pre-existing disease affecting relevant target organs (such as diabetic nephropathy or border hypertension), nutritional deficiencies (particularly of dietary cations such as iron and calcium), ethnicity, age and contact with contaminants (Centre for Disease Control Report Lead poisoning in Young Children, 2005). Higher levels or chronic exposure of lead will result in more severe symptoms such as kidney and nervous system damage (IARC, 1993), and can result from different exposure like pottery glaze, lead batteries, paint, fertilizer, gasoline and tobacco smoke etc (George Ware and M David, 2003 and World Health Organization (WHO), 1992), which can sometimes result in death.

BACKGROUND

Pb and Cd contamination constitute a major hindrance to nutritional utilization and intervention of nutrients (Wright RO, *et al* 2003; Clark N, *et al* 1988 and Chicharo JL, *et al* 1999). Constant cultivation and soil degradation has resulted in reduction in the values of food nutrients, prompting farmer and agriculturist to embank on fertilizers which is one of the major sources of heavy element contamination in rural agricultural settlements (Ceres) (Elizabeth VME, *et al* 1999). The Ceres area of the Western Cape is an agricultural town and includes communities that fully represent the South African economic spectrum and the population diversity.

AIM OF THE STUDY

The aim of this study is to investigate the presence of lead and cadmium in saliva of school children.

METHODOLOGY

Study design:

This study is aimed to assess, the levels of lead and cadmium in saliva. This technique is widely used for the determination of trace elements in matrices, especially biological materials. Analysis of lead and cadmium is done on grade one learners in primary schools in Ceres, Western Cape, South Africa.
Prior to data collection, parents of the children under investigation were asked whether they had any direct contact with objects that might increase their venerability to heavy element 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 (Table 1). Following informed consent, 62% of those asked to participates 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 lead and cadmium in saliva samples by conventional *Aqua Regia* digestion method:

The determination of lead and cadmium in saliva, 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 J, *et al* 1991). A photometric method is used in analyzing the digested samples using an atomic absorption spectrophotometer (AAS) (Unicam AAS Type solar) (Smith JC, *et al* 1979 and Vercoutere K, *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 constant optimal aspiration and furnace conditions were maintained. All machine readings were repeated twice.

SALIVA: At least 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. A total of 4 saliva samples that did have trace of contaminants were discarded. The mixed saliva was then frozen and stored in a freezer at 0°C to -4°C.

Prior to 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 filtered with Whatman no. 42 filter paper into a volumetric flask and diluted to a final volume of 100mL with DDW (Moore PD and SB Chapman, 1989). The 100mL solution was then stored in a plastic container until analysis with an AAS for cadmium and lead 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 were presented as mean, standard deviations, and Pearson Correlation Coefficients between lead and cadmium in saliva. The P values <0.01 were considered statistically significant.

Table 1: Description of study sample

Year	Total Number 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 ratio (%) of Girls/Boys
2003	544	200	115	60.0%	43/57
2004	688	226	150	64.5%	54/46
Total	1232	426	265	62.3%	49/51 Ratio 1:1

Table 2: Socio-economic characteristics of learners

		Mean Age of participants	Weight	Height	Ave numbers of persons	Household Income per month
Phase One		(yrs)	(Kg)	(Cm)	*	**
	2003	7.60	20.46	118.71	(2 - 3)	(R250 – R999)
	2004	7.84	22.48	118.62	(2 -3)	(R250 – R999)
	Total	7.73 ± 0.60	21.93 ± 4.82	118.69 ± 7.23	(2 – 3)	(R250 – R999)

*Median No. of people contributing to household income, **Average family wages range per month in Rands is (R 250 – R999) per month

Table 3: Lead and cadmium in hair and saliva samples

			Levels of trace elements in hair and saliva samples					
Parameters	**Trace element**	**No. Of Children (n)**	**Trace Element index Concentration.**	**Ref. standard Scale**	**% within standard range, below and above**			**Standard Deviation**
	*	N		**	**Within**	**Below**	**above**	
Saliva	**Lead mol/l**	249	1.07	<1.0		48	52	1.31
	Cadmium mol/l	249	0.67	0.4 – 2.25	68	22	10	0.44

*Hr_ Pb = Hair lead, Hr_Cd = Hair cadmium, Sl_Pb = Saliva lead, Sl_Cd = Saliva cadmium, (**)Ref range. (**1**), N = number of children involved in each analysis.

RESULTS

Sixty-two percent of the study sample consented to participation. This number represents 265 of the total number of 426 grade one learners over the study period (Table 1) with a male: female ratio 1:1. The mean age was 7.73 ±0.60yrs. The average weight and height of the children are 21.93 ±4.8kg and 118.69 ±7.2cm, respectively. The median household income contributors were 2 persons and that of income was R250–R999 per month (Table 2).

Table 3 shows the total number of samples collected from each participant, the average levels of lead and cadmium in saliva samples and their reference values. There were high levels of lead in saliva (1.07 ±1.31µg/L), when compared with the standard values of <1.0µg/L, saliva. The level for cadmium in saliva (0.67 ±0.48µmol/L) is also high with 56% above average but the mean is within the tolerable standard limits in the human body (< 0.55µg/L).

The Pearson correlation coefficient showed a significant correlation (P<0.01) between cadmium and lead in saliva. In other results levels of lead and cadmium were found in water and cultivated vegetables, although levels were high but within the range of reference.

Other factors such as; anthropometric data showed no significant influence on both elements as notice in both male and female pupils, although there was a significant difference in the socio-economic status of children but these could not influence the levels of lead and cadmium in saliva either, the effect on learning ability need to be investigated.

DISCUSSION

Experts agree that diagnosis and prevention of disease using saliva assay is now been accepted as a means of trace elements analysis, as more and more laboratories and medical practitioners are beginning to use this technology, especially in the field of hormonal analysis, drug testing and immunologic analysis (Schramm, *et al* 1992). Saliva analysis provides a profile of the biologically active compounds at cellular levels and is therefore a true representation of what is clinically relevant, as shown in this study. Contrary to blood analysis that only provides a profile of compounds that travel through circulatory system, mostly bound to protein. Wang et al' (Wang D, *et al* 2007) show from experiments that saliva analysis will enable one to predict, diagnose or prevent many health problems and diseases. Findings by Gonzalez *et al.* (Gonzalez M, *et al* 1997) supports this theory, and emphasized on the potential for monitoring recent exposure to ambient pollutants in blood, since circulating levels of certain polluting chemicals can be transported into salivary glands.

One of the major disadvantages of drug testing saliva is the shorter detection times of drugs compared to urine analysis – approximately 1 day compared to 3 days as a general rule. This has obvious implications for drug screening of individual suspected of drug abuse (Riet Dams, *et al* 2007 and Kato, *et al* 1993), but is not the case with heavy elements. However saliva can be very useful in heavy elements that have a longer life span and can be analyzed in the system for a very long time, this can be observed in the methodology; were prepared samples were stored over considerable period of time (Watanabe K, *et al* 2009). Other forms of non-invasive biomarker have showed different degrees of heavy elements in their analysis (AR Hashem KF Abed, 2002; AR Hashem and MR. Al-Othman, 2001).

The Centre for Disease Control and Prevention 2005 has report that, the blood-brain developed in children will be more susceptible to lead exposure and may suffer brain damage if exposed to lead contaminations (Lars J, 2003 and Jarup L, *et al* 1998). In adults this may result in lead encephalopathy, mental retardation and anemia. Long-term high cadmium exposure is reported to cause skeletal damage. The *itai-itai* (ouch-ouch) disease which is the combination of osteomalacia and osteoporosis (Nwafia WC, *et al* 2006 and Allen L. and J Casterline-Sabel, 2001) is also associated with cadmium contamination. Relatively cadmium can be attributed to low bone mineral density (osteoporosis), fractures (Kaul B, 1999 and Beard JL, 2003), cardiovascular disease (John B, 2003) and has been potentially linked to multiple human disorders (Abdulaziz A. Al-Khedhairy, *et al* 2001).

However, there is no direct relationship between concentrations in blood and saliva, mostly caused by the varying pH-values (Schramm W, *et al* 1992). In testing for drug concentration in saliva (under certain prerequisites), it is equivalent to the non-binding protein portion in plasma. They depend strongly on the pH-value of saliva and the intensity of the plasma protein binding of the drug. Therefore, it is not possible to estimate blood concentration directly from saliva. On the other hand, saliva has clear advantages with regard to the sampling procedure and the handling in the analysis. For example, saliva can be used in different sampling situations (like in a roadside survey) and should also be discussed as a pre-test for detecting drugs in suspected drivers (Magerl H and E Sculz; Stephen George and Robin A. Braithwaite, 2002). Knowledge about the distribution of drugs in plasma and in saliva is important because saliva is much easier to collect for drug analysis (Muehlenbruch B, 1982).

The aim of this pilot study was to investigate the presence of these elements, as to pre-empty further research and since this method has never been used previously hence a reference point is needed (Ogboko B, *et al* 2009). Saliva analysis can be interpreted as recent deposition of these elements, judging from the geographical location of Ceres.

CONCLUSION

The need for standard levels of measurement is needed in order to easily evaluate the effect on human exposure or deficiency which might result in nutritional based illness, sometimes due to element in-balances. It is also important to develop analytical method to monitor the levels of each elements in human especially children, as to identify any abnormality that will arise on the course of high nutritional demand resulting from consistent growth and immune response. In order to assess exposure to any abnormalities that will result from not only metabolic reaction in the body but also environmental contamination and the results of the use of noninvasive techniques in analyzing for trace element present human.

Much work is yet to be done on heavy elements analysis on saliva as against numerous once on drugs and immunologic analysis. Immunologic and forensic-toxicological analysis are originally designed and developed for urine, also very effective for saliva, is to detect and quantify exogenous substances, poisonous agents or drugs in different body fluids. Urine samples are better apt to identify active substances whereas blood samples should be used to measure the concentration of effective compounds. Despite these shortcomings saliva analysis is a promising tool. This was shown in a series of experiments with alcohol, different drugs and different measuring methods done by the Institute of Forensic Medicine in Wuerzburg confirming the usefulness of saliva substance analysis.

The reliability of trace element concentrations in saliva as index of heavy element status could be markedly enhanced when combined with other related indices measured simultaneously. Hence, the use of; blood, hair, nails and other parameters should be encouraged. In a situation where the toxic 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, heavy element binding ability make it possible to displace lower elements in the periodic table and rendering them unavailable in cell structures.

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.

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