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MANGANESE CONCENTRATION IN HUMAN SALIVA USING NAA

Hugo R. Lewgoy[1], Cibele B. Zamboni[2], Ilca M. M. A. Medeiros[2] and Jose A. G. de Medeiros [2]

[1] Universidade Bandeirante Anhanguera, UNIBAN - SP
R. Maria Cândida, 1813, Bloco G / 6°
02071-013 São Paulo, SP, Brasil
hugorl@usp.br

[2]Instituto de Pesquisas Energéticas e Nucleares, IPEN - CNEN/SP
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP, Brasil
czamboni@ipen.br
immamed@yahoo.com.br
jageiros@yahoo.com.br

ABSTRACT

In this investigation the Manganese levels in human whole saliva were determined using Neutron Activation Analysis (NAA) technique for the proposition of an indicative interval. The measurements were performed considering gender and lifestyle factors of Brazilian inhabitants (non-smokers, non-drinkers and no history of toxicological exposure). The results emphasize that the indicative interval is statistically different by gender. These data are useful for identifying or preventing some diseases in the Brazilian population.

1. INTRODUCTION

In the last years the use of saliva as a diagnostic fluid has increased. Investigations of organic and inorganic components, hormones and proteins have present significant progress for diagnostic [1,2]. Among the inorganic components Cl, K and Na are majority and responsible for maintaining the osmolarity of the saliva, while Ca, Mg, P and also Cl are responsible for maintaining the resistance of enamel to cavities. Investigations performed in saliva of periodontal patients indicate that abnormal concentration of Ca can act as an efficient indicator of periodontal diseases [3,4] while the increase of S levels can be associated with halitosis [5]. Some investigations also suggest that Mn can be used to investigate neurotoxic dysfunctions [6,7] and, although more researches are necessary to known about the absorption and retention of Manganese in the body, an investigation performed by Bouchard et al [8] offer strong support for this hypothesis.

Another aspect to be considered is the viability of using the proposition of normal range for Mn in whole saliva as a biomarker of exposure for welding professionals. A study, performed by Wang et al. [9], showed that saliva Mn concentrations of these workers (welders) can be correlated with serum concentrations with the advantage of being at a higher concentration (Mn contents are concentrated in dry saliva ~ 200 times higher then in dry serum [10]).

Recently hair had also been used for human exposure to Mn but an investigation performed by Chen and Copes [11] point out the limitations of this biological material as a biomarker, mainly due the contamination.

Considering that, saliva is less invasive to collect than blood and less prone to external contamination as hair, this biological material (whole saliva) can be a better biomarker for Mn, mainly to investigate some specific diseases. To visualize, in Fig. 1 are presented a comparison between mean values of elements concentration in whole saliva [4,5] and serum [10,12], which shows that most of the concentrations in whole saliva are higher than in serum.

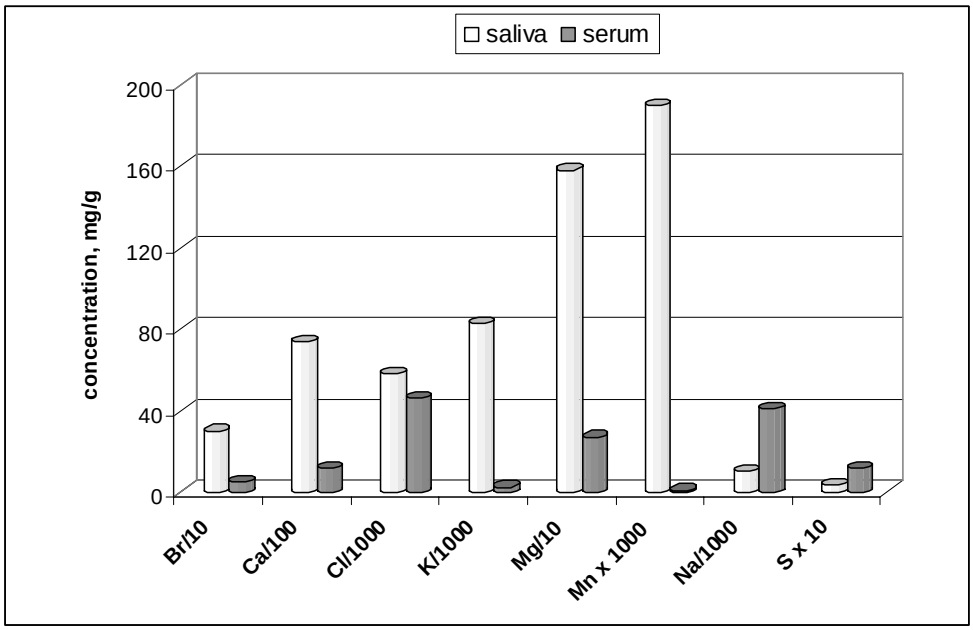

Figure 1: Mean values of the elements concentration in whole saliva and serum.

Considering the viability to optimize the use of saliva to diagnostic of some dysfunctions, recently we investigated majority elements in whole saliva [4] and now we intend to extend this investigation on to Mn concentration. We performed measurements using whole saliva because it represents better the complete oral environment when compared to saliva from individual glands [13]. Also, the quantity that can be collected is an advantage, since the average that human being can produce is approximately 0.4 mL/min [14].

In this investigation the Mn levels in human saliva were determined using Neutron Activation Analysis (NAA). The measurements were performed considering lifestyle factors (non-smokers, non-drinkers and no history of toxicological exposure) and gender of Brazilian inhabitants. The proposition of indicative interval for manganese would greatly advance in the understanding of this metal's toxicity effects.

2. MATERIAL AND METHODS

The samples of non-stimulated whole saliva were collected from 74 healthy subjects (41 women and 33 men), range from 24 to 55 years old healthy subjects. All participants were inhabitants of São Paulo city. The donors made a prior rinse with distilled water, before the collection started. The collection was performed at the same time of the day, near lunch time (at least with an interval of two hours fasting), spontaneously (without stimulation) directly in sterilized plastic containers in a dental office by a dentist. About 8-10 mL of saliva was collected from each donor. The fresh saliva was immediately frozen until to be used. For sample preparation, saliva was lyophilized (~200 - 220 mg) and transferred to a cylindrical polyethylene tube. INAA technique was used to determine the Mn content. Aliquots (50 - 100μL) of standard Mn solution (provided by Merck and Inorganic Ventures) were pipette onto Whatman $N^{\underline{0}}$ 41 filter paper and dried for few minutes using an infrared lamp.

Samples and standards were irradiated in a pneumatic station in the nuclear reactor (IEA-R1, 3.5-4.5MW, pool type) at IPEN, with a thermal neutron flux ranged from $3.21 \cdot 10^{12}$ to $6.60 \cdot 10^{12}$ n cm^{-2} s^{-1}. To determine the Mn concentration each sample and standard were irradiated for 240s. After a decay time of 600s, a gamma counting of 1800s was used to determine ^{56}Mn ($T_{1/2}$~2h, E_γ = 846keV and 1810keV). The accuracy of the standard was checked by Mn determination in the NIST 1577c. The measurements of the neutron induced activity of the sample and standard were carried out using an ORTEC GEM-60195 detector and an ORTEC 671 amplifier coupled to a MCA ORTEC 919E connected to a PC. The Mn concentration was performed using in-house software [15].

3. RESULTS AND DISCUSSION

The accuracy evaluation by Z-score for NIST 1577c standard reference material indicates the adequacy of the method is satisfactory. The determined Mn concentration (Mean Value) in whole saliva samples are presented in Table 1. All the data were obtained analyzing replicate samples. The standard deviation (±1SD), median, mode, minimum and maximum values and the indicative interval (for a confidence interval of 95%) are also presented. We also investigated the filter paper and some impurities (Al, Ca, Cl, Mg and Na) were identified but they do not interfere. This investigation was also performed in the plastic cylinder and the Ca and Mg impurities were identified, however they do not interfere.

The significance of differences between genders was assessed by Student's t-test. The level of statistical significance was taken as value of $p < 0.05$. In Fig. 2 a comparison is shown between non-stimulated human whole saliva for both genders. In Fig. 3, the Mn concentration is shown as function of gender and age. According to the Student's t-test for women (<50 years) the indicative interval is statistically different ($p < 0.05$).

Table 1: Mn saliva concentrations of the Brazilian inhabitants

Mn, μgL^{-1}	All	Men	Women
Mean Value	25	35	19

±1 SD	16	18	12
Indicative Interval	<63	<78	<56
Median	21	34	17
Mode	10	34	10
Minimum value	9	19	9
Maximum value	65	65	44

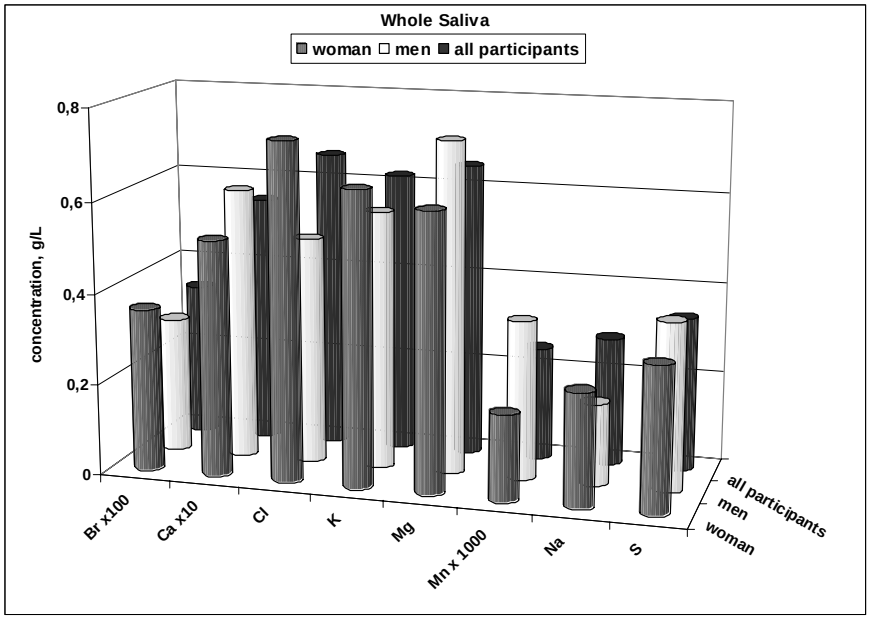

Figure 2: Mean values of the elements concentration in whole saliva

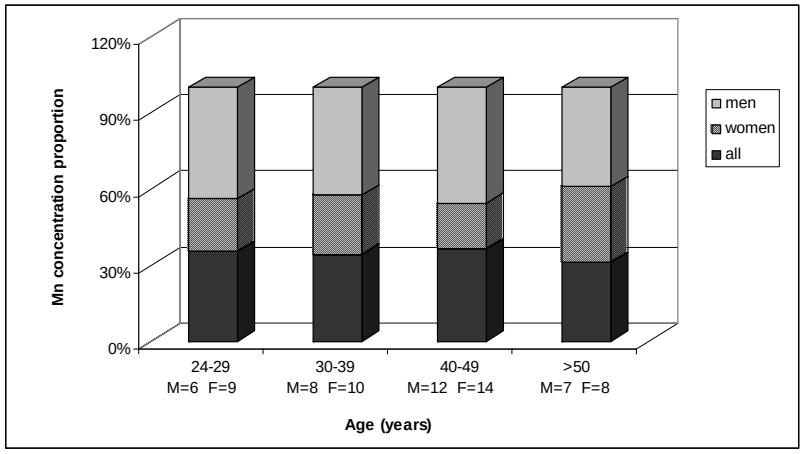

Figure 3: Proportions (%) of Mn concentration in whole saliva in function of gender and age

The data obtained can provide a scientific basis for biomedical research of some oral environment such as the regulatory mechanism of salivary secretion, which involving both volume and concentration of inorganic constituents. Recently, Mn in saliva was investigated in patient with Cystic Fribosis and the results suggest an increase when compared with the control group [16].

The Manganese also participates of several functions in body: acts in the skeletal growth and development; it is essential for glucose utilization; participates in the lipid synthesis and in the cholesterol metabolism and is also involved in thyroid hormone synthesis. Moreover, this element is a cofactor for a number of important enzyme functions (such as: cholinesterase, mitochondrial and several phosphates) and its metabolism is similar to that of iron: while the absorption in the small intestines process is slow its absorption rate is very high (~40%) [17]. Inadequate manganese intake (deficiency) has been linked to myasthenia gravis (loss of muscle strength) resulting in weak tendons and ligaments; it is also associated with possible cause of diabetes [18] while its toxicity (excess) interferes with the absorption of dietary iron, i.e., long-term exposure to excess levels may result in iron-deficiency anemia. Manganese overload is generally due to industrial pollution (industrial workers of battery manufacturing and welding professionals are at higher risk) as well as individuals undergoing inadequate supplementation [19].

Nowadays, laboratory diagnosis for Mn evaluation has been performed in serum. The present data can be useful to perform clinical practices using saliva. So, we intend to improve the statistical data for Mn in whole saliva, for all age groups, and then investigate the possible correlations with serum concentrations.

4. CONCLUSIONS

The results from the present investigation give an indicative interval for Manganese in saliva. The availability of accurate reference values for Manganese in human whole saliva represents an important indicator of the health status. The cost/benefit relation can be optimized by

making the diagnostic in whole saliva more competitive than blood (serum) analyses for some specific diseases.

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REFERENCES

1. G. Delanghe, J. Ghyselen, C. Bollen , D. van Steenberghe, B.N. Vandekerckhove, L. Feenstra, "An inventory of patients' response to treatment at a multidisciplinary breath odor clinic " *Quintessence Int.*, **30**, pp. 307-310 (1999)
2. E. Kaufman**,** I.B. Lamster "The diagnostic applications of saliva a review" *Crit. Rev. Oral Biol. Med.***13**, pp. 107-212 (2002)
3. M.E. Schutzemberger, R.T. Souza, R.E. Petrucci, M.N. Machado, V. Papalexiou, J.A. Brancher " Analise bioquímica do fluido salivar de indivíduos portadores de doença periodontal"*RSBO*, **4**(1), pp. 46 – 52 (2007)
4. H.R. Lewgoy, C.B. Zamboni, S. Metairon, I.M.M.A Medeiros, J.A.G. de Medeiros "Quantitative study of non-stimulated human whole saliva using NAA" *J. Radioanal. Nucl. Chem*, **296**, pp. 573-577 (2013)
5. C.B. Zamboni, S. Metairon, I. M. M. A. Medeiros, H. R. Lewgoy "Investigation of saliva f Patients with periodontal disease using NAA" *AIP Conference Proceedings*, **1529**, pp. 70-72 (2013).
6. R.J. Elsner, J.C. Spangler "Neurotoxicity of inhaled Manganese: public health danger in the shower?" *Med Hypotheses*, **65**(3), pp. 607–616 (2005)
7. J.G. Spangler, R. Elsner "Commentary on possible manganese toxicity from showering: response to critique". *Med Hypotheses*, **66**(6), pp. 1231–1233 (2006)
8. M.F Bouchard, S. Sauvé, B. Barbeau, M. Legrand, M. Brodeur, T.Bouffard, "Intellectual impairment in school-age children exposed to manganese from drinking water". *Environ Health Perspect.* **119,** pp. 138–143 (2011).
9. D. Wang, X. Du, W. Zheng "Alteration of saliva and serum concentrations of manganese, copper, zinc, cadmium and lead among career welders" *Toxicol Lett.* **176**(1), pp. 40–47 (2008)
10. V. Zaichick, A. Tsyb, S. Bagirov "Neutron activation analysis of saliva: application in clinical chemistry, environmental and occupational toxicology" *J. Radioanal. Nucl. Chem.* **195**(1), pp. 123-132 (1995)
11. H Chen and R. Copes. "Manganese in Drinking Water" *Environ Health Perspect*, **119**(6), pp. 240-241 (2011)
12. L. Kovacs, C.B. Zamboni, L.C. Oliveira, V.L.R. Salvador, I.M. Sato, M.R. Azevedo "Analysis of serum and whole blood using NAA for clinical investigation". *J. Radioanal. Nucl. Chem*, **278**, pp. 543-545 (2008)
13. R. Mulligan, M. Navazesh, G.J Wood "A pilot study comparing three salivary collection methods in an adult population with salivary gland hypofunction " *Spec. Care. Dent.* **15**, pp. 154-158 (1995)

14. T. Parvinen, M. Larmas "Age Dependency of Stimulated Salivary Flow Rate, pH, and Lactobacillus and Yeast Concentrations" *J. Dent. Res.* **61**(9) pp.1052-1055(1982)
15. J. A. G. Medeiros, C. B. Zamboni, G. S. Zahn, L. C. Oliveira, L. Dalaqua Jr., "Software para realização de análises hematológicas utilizando processo radioanalítico,"39º *Congresso Brasileiro de Patologia Clinica / Medicina Laboratorial*, São Paulo & Brasil, de 19 a 22 de outubro, (2005).
16. L A Candido Vieira, C. F. S. Castro, G. C. S. Feijó, L. F. Zara, A. C. B. Bezerra. "Atomic Spectroscopy Identifies Differences in Trace Element Parameters in the Saliva of Patients with Cystic Fibrosis" *Pesq Bras Odontoped Clin Integr*, **11**(2), pp. 211-216(2011).
17. Thomas L. Labor & Diagnose, 4th ed Med. Verlag Marburg 1992.
18. Kazi TG, Afridi HI, Kazi N, Jamali MK. "Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients". *Biol Trace Elem Res*. **122**(1):1-18 (2008).
19. E. W. Son, S. R. Lee, H.S. Choi, H.J. Koo. "Effects of supplementation with higher levels of manganese and magnesium on immune function" .*Arch Pharm Res*. **30(**6), pp.743-9 (2007)