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Quantitative study of non-stimulated human whole saliva using NAA

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Abstract In the past few years, the use of saliva has increased as a method for diagnosis of a variety of diseases. Investigations of flow rates, pH, molecular components, hormones and proteins have presented significant progress in clinical testing as a diagnostic tool. Inorganic elements found in saliva also have important correlations that can assist in the diagnosis of periodontal disease, but these salivary components are still poorly investigated. In this study, we investigated non-stimulated whole saliva of 44 healthy subjects and 12 patients with periodontal disease, obtained from donors at São Paulo city (Brazil). Using neutron activation analysis (NAA) technique, we found considerable metabolic changes in the salivary composition of periodontal patients: abnormal concentrations of Br, Ca, I, K, Mg and S that may be associated with periodontal, with the most effective indicator of periodontal disease being Ca concentration. The data from healthy donors also provide a scientific basis for biomedical researches of other oral diseases.

Keywords NAA · Whole saliva · Periodontal disease · Element concentrations · Diagnostics

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Introduction

Saliva is one of the most complex body fluids, consisting mainly of water (~98 %), in addition to electrolytes and enzymes. It performs several functions: it has control of the amount of body water, regulates the acidity of the mouth (pH estimates range from 6.2 to 7.7), prevents dental caries, participates in digestion and in maintaining the water balance (regulating the body fluids excretion) and also has a hormonal function, secreting a hormone that has an important role in the development of taste buds [1, 2].

In humans, there are three pairs of major glands (parotid, submandibular and sublingual) and numerous smaller glands, located mainly in the oral mucosa, contributing to saliva formation. The sub-mandibular gland contributes around 70–75 % of secretion, while the parotid gland secretes about 20–25 % and minimal amounts are secreted from the other salivary glands. Whole saliva is the mixture of these glandular secretions and other components such as bacteria and epithelial cells. While the salivary secretion is controlled by the brain in the salivary center, its flow is generated by taste [3, 4].

In the past few years, saliva usage as a diagnostic fluid has increased. Investigations of flow rates, pH, molecular components, hormones and proteins have presented significant progress for clinical testing as a diagnostic tool. Inorganic elements have shown important correlations that can be used for diagnosis, but these salivary components are still poorly investigated [4–6]. Among the inorganic constituents, Na, K and Cl are major components and responsible for maintaining the osmolarity of the saliva, while the minor components, Ca, P and Mg, in addition to Cl, increase the resistance of enamel to dental caries. The level of Ca in saliva is used as a parameter to determine susceptibility of patients to dental caries [7, 8], while the

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levels of Br and I can be used to detect inadequate ingestion of antidepressed (rich in bromides) and hormones rich in iodine (especially thyroid hormones) both highly consumed by Brazilian population [9–11].

Another common disease of the oral cavity is halitosis (oral malodor). Halitosis can be caused by dental caries, periodontal disease, as well as ulceration and necrosis [12]. In addition, impaction of food in the interproximal spaces, porous prosthesis and ill-fitting restorations [8, 13, 14] can cause halitosis as a result of microbial putrefaction [15]. These conditions can result in the release of volatile sulfur compounds (VSC), such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulfide $(CH_3)_2S$ [12, 15–17] consequently, S is also important to be quantified in saliva.

In Brazil, there is a lack of such data for human saliva. In this investigation, the goal was to determine Br, Ca, Cl, I, K, Mg, Na and S concentrations in non-stimulated human whole saliva using the NAA technique. We performed measurements using whole saliva because of the ease of collection and because it better represents the complete oral environment when compared to saliva from individual glands [18]. The major advantage for using saliva in diagnosis, comparatively to serum or plasma, is the ease of access and non-invasive method of collection. Also, the quantity that can be collected is an advantage, since the average human being can produce 1–2 L of saliva per day and the salivary flow from a healthy adult is usually approximately 0.4 mL min^{-1} [19]. Moreover, there are no special waste considerations since there is no required treatment prior to discarding samples as a regular biohazard.

We also investigated the saliva of 12 patients with a periodontal disease that has a high incidence in the Brazilian population, gingivitis, which is an inflammatory infectious disease that affects the gum also called soft tissue. Moreover, the progression of gingivitis can also affect the bone that supports teeth, also called hard tissue, which can result in bone loss or periodontitis. Periodontitis begins with the accumulation and mineralization of plaque and can lead to tooth loss [14, 20]. In this study, we intend to stimulate the multi-elemental analysis of saliva as a diagnostic tool for some specific diseases.

Experimental

The samples of non-stimulated whole saliva were collected from 44 healthy subjects (26 women and 18 men), mean age, 34.5 ± 9.6 years; range: 21–49 years and 12 patients with periodontal disease (8 women and 4 men), mean age 41.1 ± 11.2 years; range 24–56 years. All participants were inhabitants of São Paulo city. Prior to collection, the donors rinsed with distilled water. The collection was performed at the same time of the day, near lunch time, between 10.00 am to 12.00 pm (with an interval of at least 2 h fasting), spontaneously (without stimulation) and directly in sterilized plastic containers. Saliva was collected in a dental office by a dentist. Donors were stimulated to think about foods, especially citrus fruits, in order to increase the volume of salivary flux in a short of time. About 8–10 mL of saliva was collected from each donor. After collection, saliva was weighed and stored under refrigeration.

Two types of samples were prepared: 200 ± 10 μL of saliva (in duplicate) were dropped onto filter paper (Whatmann–42) using a calibrated micropipette and dried for few minutes using an infrared lamp. Also aliquots of 400 ± 20 μL of saliva were transferred to polyethylene bags and immediately frozen until used. Instrumental NAA technique was used to determine the total elemental content.

Samples and standard solutions were irradiated in a pneumatic station in the nuclear reactor (IEA-R1, 3–4.5 MW, pool type) at Instituto de Pesquisas Energéticas e Nucleares (IPEN), in a thermal neutron flux (range 6.67×10^{12}–8.42×10^{12} n cm^{-2} s^{-1}). The neutron

Table 1 Elements concentration in non-stimulated human whole saliva

Elements	MV ± 1SD	Min	Max	Range, 95 %	DL
Br, mg L^{-1}	0.34 ± 0.09 0.30 ± 0.13^{M} 0.36 ± 0.08^{W}	0.21	0.48	0.16–0.52	0.13
Ca, g L^{-1}	0.056 ± 0.017 0.060 ± 0.022^{M} 0.052 ± 0.015^{W}	0.028	0.089	0.022–0.090	0.004
Cl, g L^{-1}	0.659 ± 0.184 0.501 ± 0.197^{M} 0.739 ± 0.123^{W}	0.268	0.940	0.291–1.027	0.0009
I, μg L^{-1}	8.5 ± 9.6 3.1 ± 0.7^{M} 11.2 ± 11.0^{W}	1.9	26.0	<18.1	0.6
K, g L^{-1}	0.620 ± 0.096 0.569 ± 0.077^{M} 0.646 ± 0.098^{W}	0.455	0.767	0.428–0.812	0.022
Mg, mg L^{-1}	6.5 ± 3.4 7.3 ± 4.2^{M} 6.1 ± 3.2^{W}	1.9	13.3	<13.3	1.5
Na, g L^{-1}	0.204 ± 0.085 0.181 ± 0.066^{M} 0.215 ± 0.095^{W}	0.106	0.360	0.034–0.374	0.0008
S, g L^{-1}	0.34 ± 0.08 0.37 ± 0.10^{M} 0.32 ± 0.06^{W}	0.23	0.46	0.18–0.50	0.16

M Man, *W* Woman

irradiation condition was optimized for simultaneous determination of the elements. Irradiation times of 60 s (400 μL) and 240 s (200 μL) were used and the activated materials were γ-counted by adequate times: 120 s for Cl and Na, 900 s for Br, Ca, Mg, and S, 1,800 s for I and 1 h for K (using 200 μL of saliva) and 120 s for Cl and Na, 900 s for S and 1,200 s for Br, Ca, Mg, I and K (using 400 μL of saliva).

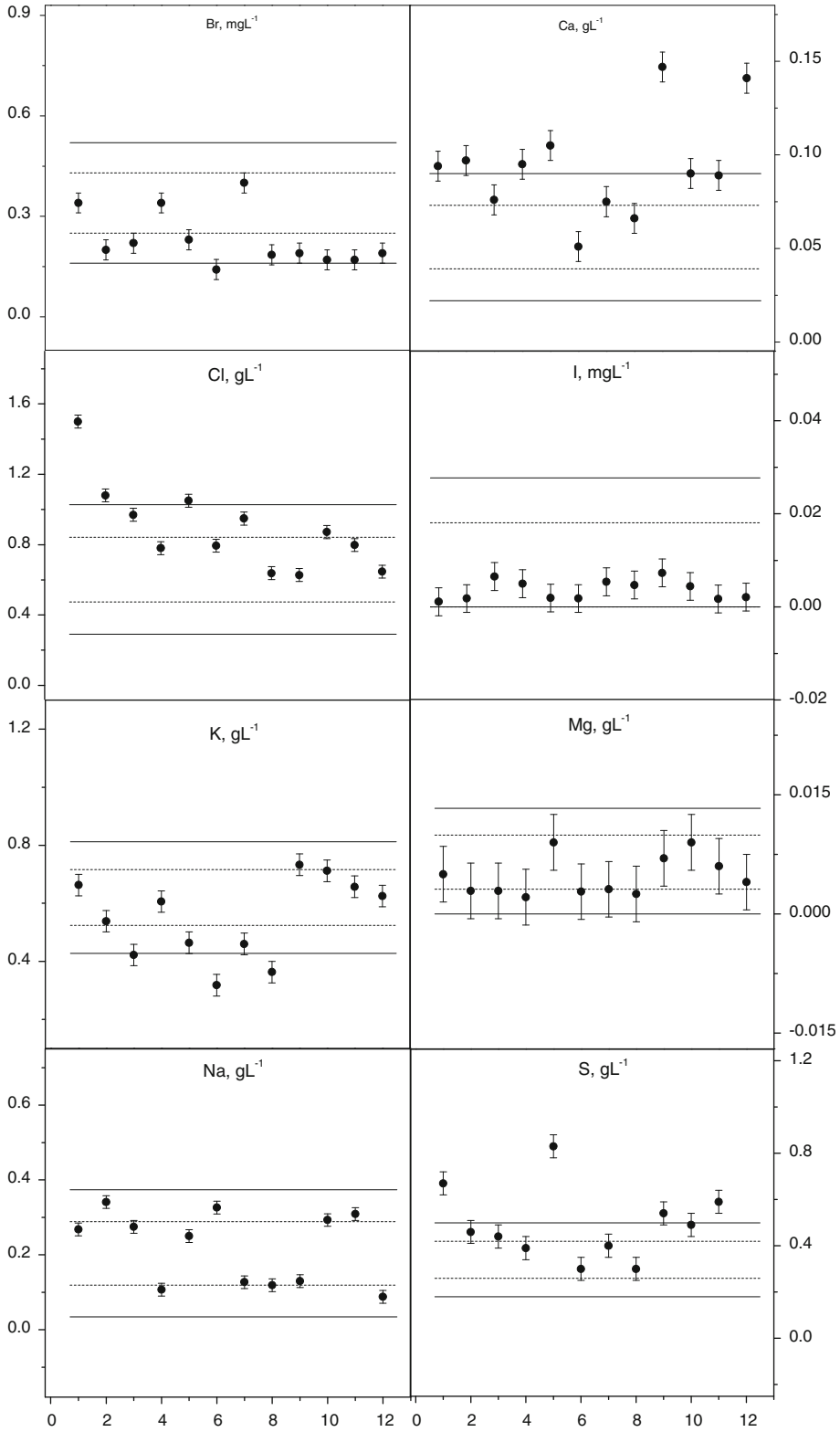

Fig. 1 The concentration of Br, Ca, Cl, I, K, Mg, Na and S in saliva samples of patients with periodontal disease. The range for the control group, considering ±1SD (*dash line*) and ±2SD (*solid line*) were also included for comparison

The γ ray measurements were performed using an ORTEC Model GEM-60195 and ORTEC 671 amplifier (in pile-up rejection mode) coupled to a MCA ORTEC Model 919E. Reference material (IAEA-A13 Blood Animal) was analyzed to verify the quality of analytical results and the Z-score values (standardized differences) obtained indicated that our results were satisfactory and are within the range of certified data at 95 % confidence level.

Results and discussion

The Br, Ca, Cl, I, K, Mg, Na and S concentrations determined in whole saliva samples from the control group are presented in Table 1. The mean value (MV), standard deviation (SD), minimum and maximum values, detection limit (DL) and range, for a confidence interval of 95 %, are also presented. The level of statistical significance was taken as value of $p < 0.05$. Differences between genders were assessed by Student's t-test and Cl was the only element for which there was a significant difference between genders. This result is consistent with data previously reported by Zaichick et al. [21], where the findings also concluded no significant differences between genders.

Figure 1 reports the results in whole saliva for 12 patients (represented by Dn, $n = 1$–12) with periodontal disease; the range for the control group, considering ± 1 and ± 2 SD, were included for comparison. According to this figure, the Na, K and Cl contents are in agreement with the normal range although for Cl about 50 % are near the uppers limits; for only one case (D1: 1.50 ± 0.04 g L^{-1}, for Cl) there is no agreement even considering the upper limit of 99 % (1.07 g L^{-1}). The results for Br, I and Mg show a decrease while for S they are near of upper limit: specifically for two patients (D1 and D5) there were significant increases in S contents suggesting also halitosis diseases [13]. For Ca there are significant differences between control and periodontal group's: most of results (92 %) are near or over the upper limit (0.090 g L^{-1}) which may be related to the periodontal disease.

Considering that one of the causes of halitosis can be associated with periodontal disease [12], the behavior of Ca and S concentration ratio in these patients were also investigated and they can be observed in Fig. 2. This figure shows the effects of Ca:S (C_{Ca}:C_S) in patients with periodontal disease in comparison to ratios for the control group. These results are consistent with several other studies [4, 6–8, 13], which have also suggested that high Ca (mainly) and S levels in whole saliva can be associated with periodontal disease.

Saliva has other properties that may be useful in the diagnosis of oral disorders, among them, the regulatory

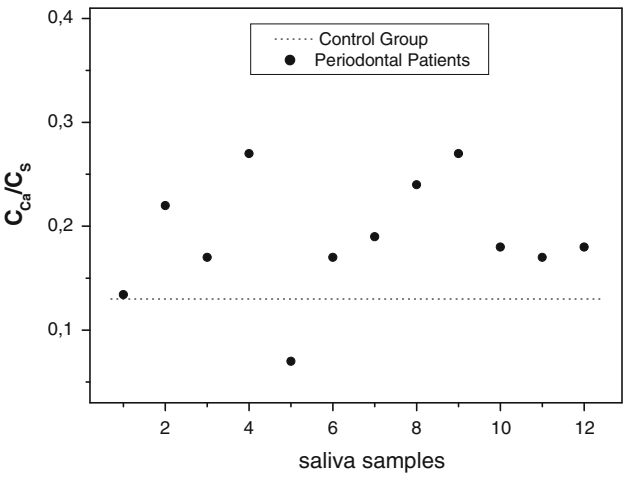

Fig. 2 Concentration ratio comparison between C_{Ca}:C_S for control and periodontal disease group

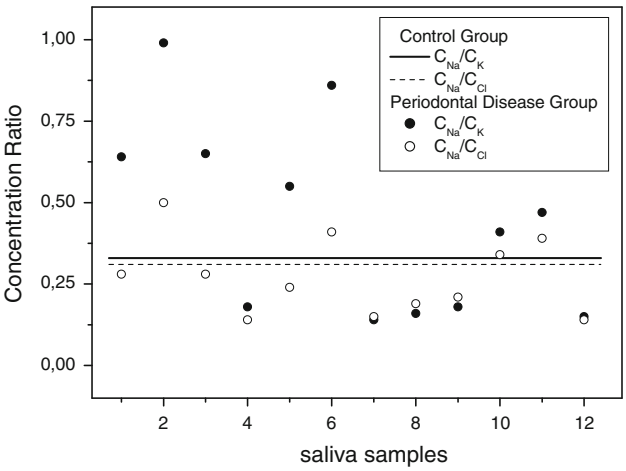

Fig. 3 Concentration ratios comparison between C_{Na}:C_K and C_{Na}:C_{Cl} for control and periodontal disease groups

mechanism of salivary secretion. This involves both volume and concentration of the major inorganic constituents (Na, K and Cl). The concentrations of these elements are dependent on the rate of salivary secretion. Normally, the secretion rates (in whole saliva) of Na:K (C_{Na}:C_K) and Na:Cl (C_{Na}:C_{Cl}) are practically constant and estimated at approximately 0.3 [22]. Our results for the control group agree with these concentration ratios with an average of 0.33 for C_{Na}:C_K and 0.31 for C_{Na}:C_{Cl} in the control group. But, the periodontal disease group had more variation in these ratios. Figure 3 presents the effects of C_{Na}:C_K and C_{Na}:C_{Cl} in patients with periodontal disease in comparison to ratios for the control group.

According to Fig. 3, the effect of the periodontal disease is accentuated in C_{Na}/C_K concentration ratio, i.e. 58 % of the estimates are very high. Related to C_{Na}/C_{Cl} concentration ratios of the periodontal patients the estimates are

near or lower (in 67 %) when compared with the control group.

Conclusion

The availability of accurate reference values for inorganic elements in human whole saliva represents an important indicator of health status. These data provide a scientific basis for biomedical research of oral diseases when compared to established concentrations obtained from healthy donors.

Investigations performed in whole saliva of periodontal patients, indicate that abnormal concentrations of Br, Ca, I, K, Mg and S may be associated with periodontal disease and that Ca concentration can be used as an effective indicator for periodontal diseases. Also, the cost/benefit ratio makes saliva as a diagnostics tool more competitive than blood analyses for specific diseases.

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