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Investigation of ions in human whole saliva by analytic techniques

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Abstract. In this study, ions of clinical relevance in non-stimulated human whole saliva obtained from healthy subject's donors (adults and children) at São Paulo city (Brazil), were investigated. The Instrument Neutron Activation Analysis (INAA) and Energy Dispersive X-Ray Fluorescence (EDXRF) techniques were used. The comparison concentration between adults and children for Cl, K, Ca and Fe showed significant differences for all elements, emphasizing the need of adopting different reference values.

1. Introduction

Saliva is a body fluid comprising 95 % of water and 0.5 % of ions and molecules. This fluid has multiple functions, such as antibacterial, antiviral and antifungal action. It also performs the control of the quantity of water of the organism, the maintenance of the acidity of the mouth (which prevents tooth decay), the maintenance of the balance that regulates the excretion of body fluids, as well secretes hormones that play an important role in the development of the palate. In the past years, the use of saliva as a diagnostic fluid has presented significant progress in clinical testing of several diseases [1-3]. The main advantage of using saliva as a diagnostic tool is that the biomarkers present in serum and urine are also found in saliva. In addition, it is easy and quick to collect (non-invasive procedure).

The salivary secretion occurs into the salivary glands (in a flow around 1.5 ml/min) from parotid (~50 %), submandibular (~ 35 %), sublingual (7 – 8 %) and from several smaller glands (< 7 %). The whole saliva is the mixture of these glandular secretions and other components (such as bacteria and epithelial cells) and its collection can be done using stimulating agents or without stimulation. The availability of accurate reference values for inorganic elements in human whole saliva represents an important indicator of the health status. Nowadays, the evaluation of ions in saliva can identify drug intake, cardiovascular dysfunctions and problems in the thyroid gland [1,2]. Studies with the Brazilian population (adults) have already been performed using Neutron Activation Analysis (NAA) and X-Ray Fluorescence (XRF) techniques generating promising results for the diagnosis of oral diseases [4-6]. Now we intend to investigate saliva obtained from adults and children. In this study, non-stimulated human

whole saliva obtained from healthy subject's donors (adults and children) at São Paulo city (Brazil), was investigated. The Instrument Neutron Activation Analysis (INAA) and Energy Dispersive X-Ray Fluorescence (EDXRF) techniques were used. The elements Cl, K, Ca and Fe were selected because they can be used as biomarkers: Ca is used as a parameter to determine the susceptibility of dental caries; Cl, Ca and Fe, increase the resistance of enamel to caries; K and Cl are responsible for maintaining the osmolarity and Fe can be a predictive marker of iron deficiency [5,7-9].

2. Experimental Procedure

SAMPLE PREPARATION**:** saliva obtained from donors, adults (18 – 60 y) and children (2 – 17 y) at São Paulo city (Brazil). Adults (20 samples): ~ 2 mL were collected in dental office by a dentist directly in sterilized plastic containers. For children (15 samples): ~ 1 mL was collected in pediatric hospital (HU - USP). The samples were lyophilized. Considering that the INAA and EDXRF procedures are non destructive, the same sample was used for both analyses.

INAA: Samples of adults and standard solution (Cl, K, Ca and Fe) were irradiated at IEA-R1 (4.5 MW, pool type) nuclear reactor at IPEN in a thermal neutron flux, for minutes to hours, and gamma counting using HPGe (GEM-60195) detector connected to a MCA ORTEC -919E.

EDXRF: Samples (adults and children) were excited and measured using a portable X-Ray Spectrometer: Ag X-Ray target and Si Drift detector (25 mm^2 x 500 μm / 12.5 μm Be window) and counting time of 300 s measured with 30 kV and 5 μA. The spectrometer was calibrated for Cl, K, Ca and Fe using linear regression method for concentration up to 500 ppm.

3. Results and Discussion

The elements concentrations determined in non-stimulated whole saliva samples are presented in Table 1 (adults) and Table 2 (children). The results were expressed by: Mean Value (MV), Standard Deviation (± 1 SD), Minimum (min) and Maximum (max). Figure 1 was elaborated to show the comparison between whole saliva samples of adults and children. The data was subjected to a statistical analyses by the students *t-test.*

Table 1. Elements concentration in adults non- stimulated whole saliva

Elements mg/kg	MV	± SD	Min	Max
Cl				
EDXRF	776	299	350	1332
INAA	620	168	290	1030
K				
EDXRF	817	278	406	1417
INAA	612	90	299	702
Ca				
EDXRF	131	62	54	247
INAA	112	34	64	224
Fe				
EDXRF	151	47	77	286
INAA	193	81	49	201

Table 2. Elements concentration in children non-stimulated whole saliva by EDXRF

Elements, mg/kg	MV	±1SD	Min	Max
Cl	497	303	62	941
K	495	284	91	944
Ca	57	41	17	138
Fe	85	32	42	153

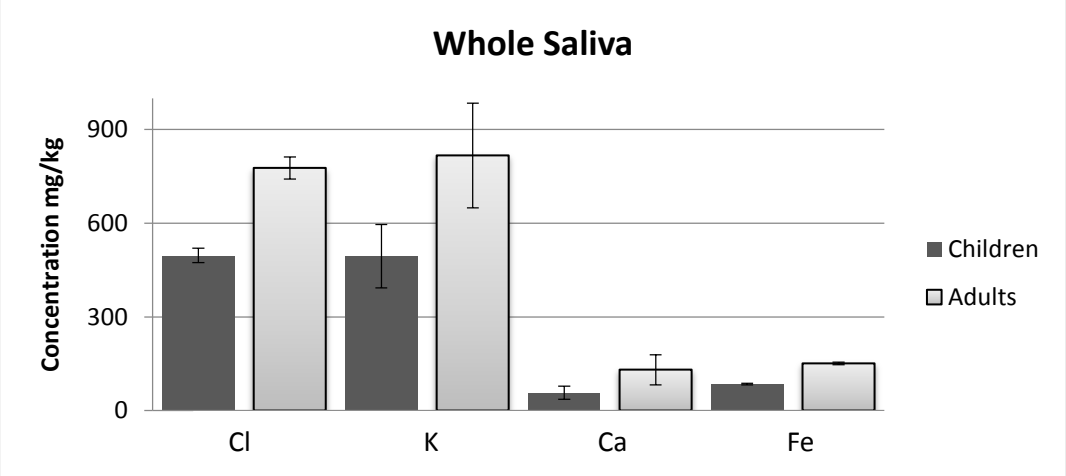

Figure 1. Comparison between the inorganic elements, in whole saliva samples of adults and children

According to the Student's *t-test* the comparison between the INAA and EDXRF techniques (Table 1) showed the discrepancy of results to be insignificant at 95 % confidence level for all element while the comparison concentration between adults and children (Table 2) emphases statistically differences ($p<0.05$) for all elements. However, more large-scale studies are needed to establish the normal range for these elements in saliva aiming its application in biochemistry tests.

Through these investigations, we also found that portable XRF spectrometer is suitable for the rapid and highly sensitive analysis of saliva.

4. Conclusion

The reference values presented in this study emphasizing the need of adopting different recommendations of specifics ions (Ca, Cl, Fe and K) in whole saliva for adults and children.

5. Acknowledgments

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