

## Investigation of Fe and Ca in non-stimulated human saliva using NAA

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**Abstract.** In this study we investigated non-stimulated human whole saliva of healthy subjects and patients with periodontal disease using Neutron Activation Analysis technique (NAA). The measurements were performed in the IEA-R1 nuclear reactor at IPEN-CNEN/SP. We found considerable metabolic changes mainly in Fe and Ca concentration in whole saliva of periodontal patients. These data are useful for identifying or preventing this oral disease in the Brazilian population.

### 1. Introduction

The use of non-stimulated human whole saliva has been presenting a significant progress in clinical testing for diagnostics of several diseases (cancer, diabetes, autoimmune disorders, cardiovascular problems, viral and bacterial infections and also in psychiatric therapy) [1]. The major advantage for using saliva in diagnosis, comparatively to serum or plasma, is the easy access and facility of collection (non - invasive) as well as the quantities that can be collected (a healthy adult can produce about 0.4 mL saliva/min). According to the latest data on oral health in Brazil (INCA- Incidence of Cancer in Brazil, 2014) [2] the prevalence of oral disease has increased in the last years and varies by regions, gender and age groups: while in the South and Southeast regions there are prevalence of oral cancer for adult men (52%) compared with all cases in the country, the North Region has higher proportion of periodontal disease index of 66 % for the age group 15 to 19 years compared with the national average of 54%. The periodontal disease begins with the accumulation and mineralization of the plaque and can lead to tooth loss [3]. Recent investigations suggest that some inorganic elements, mainly Ca and S contents in non – stimulated saliva can be biomarkers of this disease [4,5]. In this study we intend to compare the behaviour of Fe and Ca in whole saliva of healthy subjects (control group) and periodontal patients using Neutron Activation Analysis (NAA) technique. We aim to stimulate the biochemical analysis of whole saliva as a diagnostic fluid, mainly for periodontal disease that has high incidence in Brazilian subjects.



## 2. Experimental Procedure

The samples of non-stimulated whole saliva were collected (in duplicate) from 38 healthy subjects and 12 subjects with periodontal disease, male adults, and range from 39 to 57 years old. All participants were inhabitants of São Paulo city, where there is prevalence of this oral disease [2]. The donors made a prior rinse with distilled water, before the collection. 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 3 - 4mL of saliva was collected and lyophilized. Samples ( $400 \pm 5\% \mu\text{L}$ ) and standards (aliquots of standard solutions) were irradiated for 4 h in the nuclear reactor (IEA-R1, 4.0-4.5MW, pool type) at IPEN, with a thermal neutron flux that ranged from 3 to  $5 \cdot 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ . After a decay time of several days, a gamma counting of 8 hours was used to determine  $^{47}\text{Ca}$  ( $T_{1/2} \sim 4.5 \text{ d}$ ,  $E_{\gamma} = 1297 \text{ keV}$ ) and  $^{59}\text{Fe}$  ( $T_{1/2} \sim 44 \text{ d}$ ,  $E_{\gamma} = 1099 \text{ keV}$ ). The element Ca was also determined by  $^{49}\text{Ca}$  ( $T_{1/2} \sim 9 \text{ min}$ ,  $E_{\gamma} = 3084 \text{ keV}$ ) using short time irradiation (300 s) and counting time of 1200 s. 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 concentrations were performed using in-house software [6].

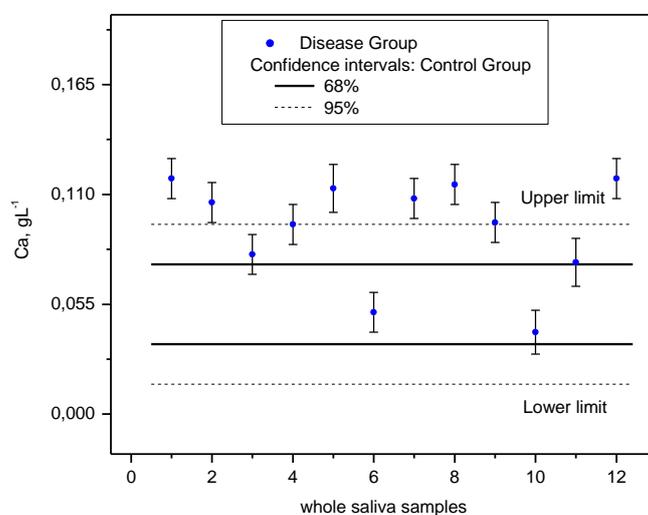
## 3. Results and Discussion

The Ca and Fe concentrations in whole saliva of healthy subjects (control group) are showed in table 1. The mean value (MV), standard deviation (SD), minimum value (Min), maximum value (Max) and reference interval (Range, for a confidence interval of 95%), are presented.

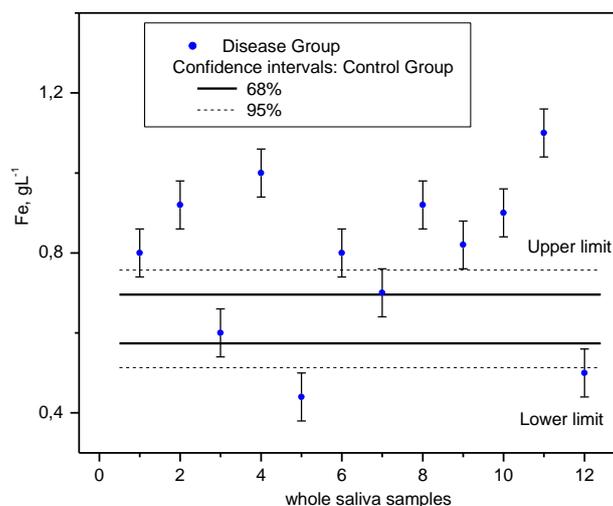
**Table 1.** Ca and Fe concentrations in non-stimulated whole saliva of healthy subjects.

Elements	MV	$\pm 1\text{SD}$	Min	Max	Range
					(95%)
Ca	0.055	0.020	0.028	0.079	0.015 – 0.095
	0.056 [4]	0.017			
Fe	0.635	0.061	0.211	0.822	0.513 – 0.757
	0.607 [7]	0.036			

To visualize in figures 1 and 2 are showed the concentration and associated uncertainty of Ca and Fe concentrations, respectively, in patients with periodontal disease; the standard deviation ( $\pm 1$  and  $\pm 2$ ) for the control group were included for comparison.



**Figure 1.** Ca concentrations and associated uncertainty of periodontal disease group.



**Figure 2.** Fe concentrations associated uncertainty of periodontal disease group.

The results from this investigation give an indicative interval for Ca (0.015 - 0.095 g/L) in agreement with recent data [4]. Particularly, for Fe, the present result (0.513 - 0.775 g/L) is the first estimative for Brazilian population and it is in agreement with data from other population [7].

The effect of the periodontal disease (Figures 1 and 2) emphasizes the increase in Ca and Fe concentrations comparatively to healthy group. According to the Student's t-test the Ca and Fe concentration in subjects with periodontal disease are statistically different ( $p < 0.05$ ) of health group for most of the case. Although Ca is usually used as an effective indicator of periodontal disease [1, 3-5], the similar behaviour of Fe suggests that its estimation in whole saliva can also be used as biomarker.

Another aspect to be considered is the relationship between proteins and inorganic elements. Levels of Ca and Fe are also important for keeping the health status of the tooth: while Fe present in lactoferrina protein participates in the mechanism regulating of saliva production (protecting the teeth of its demineralization), Ca present in some proteins (estaterina, histatin and proline) participates in the mechanism of production of Hydroxyapatite crystal (i. e. alterations can inhibits the growth of the tooth).

#### 4. Conclusion

The increase of Fe in whole saliva of subjects with periodontal disease suggests that this element can be an efficient monitor of this oral disease as well as Ca. Furthermore, the data of the present investigation provide scientific basis for biomedical research of other oral diseases using the levels evaluation of these inorganic elements in human whole saliva.

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