Secretory IgA and salivary flow in smokers and non-smokers

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Abstract

Objective: To compare the concentration levels of secretory immunoglobulin A (SIgA) and salivary flow between smokers and non-smokers.

Methods: The sample was recruited at the Dentistry School of the Pontifical Catholic University of Rio Grande do Sul. The participants were asked to spit into the tubes for five minutes (unstimulated saliva). The tubes containing the samples were immediately weighed to calculate the salivary flow through the gravimetric method. Single radial immunodiffusion plates were used as well as a graduated magnifying glass specific for reading the bands of the precipitation.

Results: The sample comprised 47 individuals: 22 smokers who smoked 30 cigarettes or more per day for more than 10 years, and 25 non-smokers, who composed the control group. The Mann-Whitney test ($P < 0.05$) found no significant difference between the groups with regard to either the concentration of the SIgA or the salivary flow. The median value of the salivary flow was 0.73 among the smokers and 0.70 among the non-smokers, and the median for SIgA was 10.20 among the non-smokers and 7.93 among the smokers.

Conclusion: There were no significant differences in the levels of SIgA and salivary flow between the smokers and the non-smokers in the investigated sample.

Keywords: Saliva; immunoglobulin A, secretory; tobacco

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IgA secretora e fluxo salivar em indivíduos tabagistas e não tabagistas

Resumo

Objetivo: Comparar os níveis de imunoglobulina A secretora (SIgA) e fluxo salivar em indivíduos tabagistas e não tabagistas.

Método: A amostra foi selecionada na Faculdade de Odontologia da Pontifícia Universidade Católica do Rio Grande do Sul. O paciente foi orientado a expelir a saliva durante cinco minutos (saliva não-estimulada). Os tubos contendo as amostras foram imediatamente pesados para avaliação do fluxo salivar pelo método gravimétrico. Foram utilizadas placas de imunodifusão radial simples e uma lupa graduada específica para a leitura dos halos de precipitação.

Resultados: A amostra foi composta por 47 indivíduos: 22 indivíduos tabagistas, consumidores de 30 cigarros ou mais diariamente, por um período superior a 10 anos e um grupo controle com 25 indivíduos não tabagistas. Utilizando o teste de Mann-Whitney ($P < 0,05$), verificou-se não haver diferença estatisticamente significativa, para os grupos de indivíduos tabagistas e não tabagistas, nos valores de SIgA e fluxo salivar. A mediana para fluxo salivar foi de 0,73 nos indivíduos não tabagistas e de 0,70 nos tabagistas e para SIgA foi de 10,20 para não tabagistas e de 7,93 para tabagistas.

Conclusão: Na amostra estudada não houve diferença significativa nos níveis de IgA secretora e fluxo salivar de indivíduos tabagistas e não-tabagistas.

Palavras-chave: Saliva; imunoglobulina A secretora; tabaco

Introduction

Saliva acts in deglutition and phonation, and due to its composition, it also participates as a lubricant in the formation of the bolus. It also acts in the defense against virulent microorganisms, aiming at the protection of the oral cavity and the maintenance of the dental integrity. Therefore any change affecting the saliva might compromise the integrity of the oral soft and hard tissues [1].

According to Sreebny [2], salivary dysfunction is indicative of systemic and oral diseases. Saliva is increasingly used for diagnostic purposes, and several easy-to-perform salivary tests are already available for the identification of oral or systemic diseases.

From the perspective of its functional activities secretory IgA (SIgA) is considered to be the organism main mechanism of local defense and it is also considered to play an important role against the penetration of microorganisms and allergenic substances inside the organism. Although the studies on SIgA tend to focus on its cariogenic potential some authors have also assessed its activity with regard to periodontal disease, opportunistic infections, and oral cancer [3].

According to Taybos [4], smoking affects the epithelial surface resulting in changes in the oral tissues. Several changes may therefore occur including increased pigmentation, epithelial thickening, gingival recession, and dental abrasion. In addition to impairing the functioning of the salivary glands, smoking also increases the risk for periodontal disease and oral cancer.

Data collected in studies conducted with smokers might contribute to the prevention of diseases since understanding the relationship between smoking and the changes it causes in the oral tissues might allow for prevention or early treatment.

Precise diagnostic methods are essential tools, especially for the prevention of diseases affecting the stomatognathic system. Saliva is a bodily fluid that is easy to collect, and the costs of the tests for saliva are usually lower than they are for tests using serum or urine.

Therefore, given the need for further research and information on the components of saliva and the changes that these components undergo relative to extrinsic factors (e.g., smoking), the aim of the present study was to investigate the effects of smoking on SIgA concentration and on salivary flow by comparing a group of smokers to a group of non-smokers with similar characteristics.

Methods

This study was approved by the Committee of Ethics in Research of the Pontifical Catholic University of Rio Grande do Sul (RS) (08/04479) in accordance with national and international guidelines and regulations of the Declaration of Helsinki.

The study was conducted with samples of unstimulated whole saliva collected from 22 individuals of different ages who were smokers and had consumed 30 cigarettes or more per day for more than 10 years. The age range of the volunteers was between 25 and 50 years old.

Twenty-five non-smokers were selected to compose the control group.

To be included in the study, the participants were given a detailed description of the procedures, and they agreed to participate by signing an informed consent form.

Individuals younger than 19 years old and individuals with chronic disorders or who were using systemic medications that might interfere with the results were excluded from the study. Furthermore, participants who had been subjected to radiotherapy or who had been irradiated in the previous six months were also excluded from the sample.

Saliva collection was performed at the outpatient clinics of the School of Dentistry of the Pontifical Catholic University of Rio Grande do Sul (Pontifícia Universidade Católica do Rio Grande do Sul – PUCRS).

Before the collection of the samples, the participants responded to a questionnaire on oral hygiene and dental conditions. At that time the participants were informed that they must not drink or eat for two hours prior to the collection of saliva.

The materials used to perform the test included sterile tubes that had been previously weighed using precision analytical scales (AG 204 Mettler Toledo, Barueri, São Paulo, Brazil). The saliva was collected from 7:30 to 10:30 in the morning to rule out interference by the circadian cycle. Before the collection of the samples, the participants were asked whether they had refrained from food and fluid intake, as previously instructed. The participants sat on a common chair and were asked to spit into a tube for five minutes (unstimulated saliva).

The tubes containing the samples were weighed immediately. The salivary flow was measured by means of the gravimetric method, whereby the flow speed is calculated as the difference in the flask weight before and after the saliva collection, where one gram of weighed saliva corresponds to one mL of saliva produced.

The samples were stored at a temperature of -15 ºC until the time of analysis and, before they were subjected to laboratory testing, they were centrifuged to separate desquamated epithelial cells, bacteria, blood cells, and food residues. The salivary IgA was assessed by means of single radial immunodiffusion, where immunocomplexes are formed with specific antibodies in agarose gel. These complexes become visible as precipitin rings with the diameters proportional to the concentration of IgA in the sample.

Single radial immunodiffusion allows for the measurement of the amount of antigen. The antibody is added to agarose dissolved in water which is then placed on slides and left to solidify. Holes are cut into the resulting gel to add a standard volume of test antigen at various concentrations. The plates are left to rest for 24 hours, and during this period, the antigen diffuses outwards and forms soluble complexes (with an excess of antigens) with the antibody. These complexes keep on diffusing and binding more antibodies until they reach the equivalence point, when they

precipitate, forming rings. The internal area of the precipitin ring, which is measured as the square of the ring diameter, is proportional to the antigen concentration. The unknown data are extrapolated from a standard curve [5].

Single radial immunodiffusion plates (LC-Partigen-IgA, Dade Behring, Marburg, Germany) and a graduated magnifying glass that is specific for reading bands of precipitation in single radial immunodiffusion were used to perform the test. To assess the accuracy of the measurements, a commercial control sample, N/T protein control SL/H – CRM 470 with an average value of 6.5 mg/dL (ranging from 5.5 to 7.5 mg/dL) (Dade Behring, Marburg, Germany), was used.

For the analysis of the data, first the Shapiro-Wilk normality test was used (at the 5% significance level) because in case the variables did not exhibit the conditions of normality they would be subjected to the (non-parametric) Mann-Whitney test.

Results

The initial analysis of the data by means of the Shapiro-Wilk test at a 5% significance level showed that the variables SIgA and salivary flow (saliva/min) did not meet the normality assumption. In addition to this the variability around the mean was quite high, making this parameter a poor predictor. Therefore a non-parametric test was used, i.e., the Mann-Whitney test, to achieve a more reliable analysis of the data.

Using the Mann-Whitney test at a 5% significance level, there was not a significant difference in the SIgA concentration and in the salivary flow (saliva/min) between smokers and non-smokers (Table 1, Figures 1 and 2).

Discussion

The results of the present study indicated no significant difference in the levels of SIgA and in the salivary flow between smokers and non-smokers. These results might be attributed to the relatively small sample size and also to the number of cigarettes, as consumption of 30 cigarettes per day might not be sufficient to induce changes in the investigated variables. Migliari and Marcucci [3] also compared the concentration of SIgA in unstimulated whole saliva between smokers and non-smokers, and the results showed a reduction of the SIgA concentration in the saliva of the individuals who smoked 40 or more cigarettes per day. Those authors concluded that the high consumption of cigarettes might cause an immunodepressant effect on the levels of IgA in the saliva. However, similarly to the present study, those authors did not find a significant difference in the salivary flow between the investigated groups.

Table 1. Comparison of the concentration of secretory IgA and salivary flow between smokers and non-smokers.

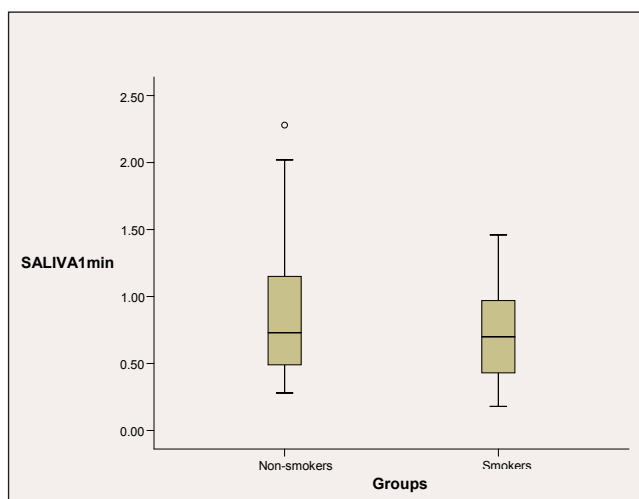| | Group | | | | | | P |
| | Non-smokers | | | Smokers | | | |
	Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75	
Saliva/min	0.73	0.47	1.19	0.70	0.43	1.01	0.488
SIgA	10.20	5.87	13.20	7.93	4.56	11.00	0.224

Fig. 1. Comparison of the salivary flow between smokers and non-smokers.
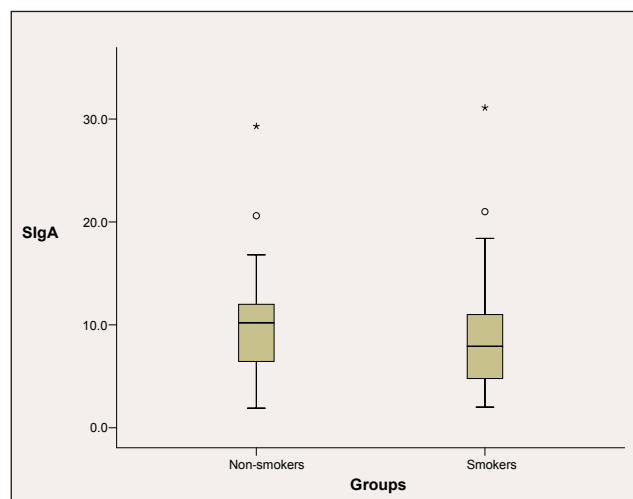

Fig. 2. Comparison of the secretory immunoglobulin A levels between smokers and non-smokers.

According to Bennet and Reade [6], the immunodepressant action associated with the consumption of cigarettes does not occur directly on the salivary glands, but it most likely results from the action of the products of the combustion of tobacco. These tobacco consumption products once absorbed into the bloodstream act on the salivary gland cells or on the immune system cells that are involved in the production of IgA. The salivary flow is the main factor involved in the variation of the concentration of IgA and the other salivary components; the concentration of IgA raises with the increases in the salivary flow. In a study conducted by the abovementioned authors, the concentration of IgA was smaller in the group of smokers compared to the control group. According to the authors, the results indicate that the regular consumption of a large number of cigarettes might cause damaging effects to the mechanisms of synthesis and secretion of SIgA, resulting in reduction of the salivary levels of that antibody.

Other factors might also alter the production of immunoglobulins as the synthesis and secretion of IgA depends not only on antigenic stimulation but also on neuroendocrine control. Thus changes in the neuroendocrine function, such as the ones induced by stress, exercise, medications, the menstrual cycle, and pregnancy, might affect the SIgA levels [7-8].

Rockenbach et al. [9] analyzed SIgA, calcium and phosphate concentrations, pH and salivary flow by comparing unstimulated saliva from pregnant and non-pregnant women and found a significant difference between the groups with regard to the levels of SIgA and the pH. The saliva of the pregnant women exhibited a lower pH and a greater concentration of SIgA compared to the non-pregnant women saliva. However no significant difference was found in the salivary flow or the calcium and phosphate concentration between these two groups. Hormonal changes may be related to the SIgA levels that are found among the pregnant women as the production of estrogen and progesterone increases gradually until the eighth month of pregnancy, and these hormones modulate the immune system during pregnancy [10].

Thus the concentrations of IgA found were not only influenced by the cigarette consumption but also by other factors, as indicated by the abovementioned authors. And the number of cigarettes daily taken seems to be the main factor related to the damage caused to the smokers' organism.

Some systemic diseases impair the functioning of the salivary glands, thus modifying the amount and quality of the saliva due to alterations in the chemical components and in the physical properties of the saliva. These salivary changes may serve as markers, allowing for the saliva to be used as a diagnostic test in some diseases [11]. For that reason, individuals with chronic disorders or who are using systemic medications, as well as those subjected to radiotherapy or who have been irradiated in the previous six months, were excluded from the present study.

It is worth emphasizing the importance to researchers of diagnostic tests that use saliva, because it is easier to be collected than blood. In addition, advanced techniques and devices for instrumentation and chemical analysis have resulted in the increasing use of this technique [12-13].

According to Lawrence [14], in the past, serum was the fluid most frequently used for the diagnosis of diseases; however, saliva exhibits many advantages when compared to serum and urine. The saliva is relatively easy to collect in amounts that are sufficient for tests at the clinical and the laboratory setting, and the cost tends to be lower when compared to the use of serum and urine. Salivary tests for antibodies (against viruses and bacteria), unconjugated steroid hormones (estrogen, testosterone, progesterone), environmental toxins (cadmium, lead, mercury), tobacco (nicotine), and some drugs (ethanol, lithium) are sufficiently sensitive to accurately measure the concentrations of such substances in the saliva, similarly to the tests using blood.

Currently, fluids with saliva are used in the diagnosis of certain diseases or disorders. The ability to measure several molecular compounds in the saliva and to compare them to serum compounds have made its use viable in the study of microorganisms. Technological advances have allowed for the analysis of saliva, not only with regard to oral health but also to collect information on the overall health of patients. The tests using saliva as well as the tests using blood have two main goals, i.e., to identify individuals with systemic diseases and to follow up the progression of treatment in patients [15].

Tang et al. [16] used unstimulated saliva to establish the pattern of salivary protein expression in adults with periodontitis. The diagnostic method chosen was electrophoresis in sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE), and the results showed that the 18 kD, 15 kD and 13 kD protein bands might strongly correlate with the occurrence of periodontitis.

Saliva has also been used to monitor the risk for caries as it has been considered to be a useful biological medium due to its buffer capacity. Currently, it is being investigated in detail as a tool for the diagnosis of systemic diseases that alter its composition and interfere with the function of the salivary glands, e.g., Sjögren's syndrome, alcoholic liver disease, cystic fibrosis, sarcoidosis, diabetes mellitus, and adrenal cortex diseases. The polymerase chain reaction (PCR) method is used in oral fluids as a transmitter of microbial DNA in the detection of viral infections, such as the Kaposi's sarcoma-associated herpesvirus, and bacteria, such as *Helicobacter pylori,* which are associated with gastritis, peptic ulcer, and possibly also with stomach cancer [16-17].

The relevance of diagnosis when using saliva as the tested material is discussed by several studies that used it to assess specific components and determinants for the purpose of discovering diseases [14,18-20]. Searching for the relation between oral and overall health, research is increasingly focusing on the use of saliva in tests for the diagnosis of systemic diseases and the monitoring of overall health. The reason behind such interest is the facility to collect and to perform this novel diagnostic option, where several technologies are used to recognize a variety of

salivary components that might serve as biomarkers to identify changes in the health of individuals. Such salivary tests are attractive for patients because they are non-invasive and easy to perform when compared to other procedures. In addition, they are marketed as diagnostic kits that allow patients to monitor their health status by themselves [12].

The present study used unstimulated saliva because it reflects the natural conditions in the human body without the use of laboratory artifacts. Unstimulated saliva plays an important role in the maintenance of the oral health and is predominant during sleep and most daytime activities; in addition to this it is present in the oral cavity for approximately 14 hours per day.

The gravimetric method was chosen to measure the salivary flow whereby the difference in the flask weight, before and after the saliva collection, provides the flow speed because the weight, rather than the volume, provides the most accurate measurement of the amount of saliva that is produced [2,11,21]. As the circadian cycle and food intake are factors that might influence the salivary flow and also its physiological characteristics, they were taken into account and standardized for the collection of saliva in the present study [14].

Although the present study did not find a significant difference between the salivary variables of smokers and non-smokers controls, the importance of considering the damaging effects of tobacco must be emphasized as several published studies report on its negative characteristics. This includes the study by Zamboni [22], which stresses the carcinogenic ability of tobacco smoke and that smoking is the main factor related to lung cancer. Smoking also increases the risk of mouth, larynx, esophagus, bladder, pancreas and kidney cancer, whereas cervical and gastric cancer also seems to be influenced by smoking. All of these neoplasms will increase among women as a function of their increasing smoking rates. Smokers are also known to be more prone to cardiovascular complications among other illnesses.

Reznick et al. [23] showed that the smoke of cigarettes destroys several salivary components, including protective components such as peroxidase which is the most important antioxidant enzyme.

Therefore the continuous improvement of laboratory techniques and the use of standardized methods and also the use of representative and appropriate samples in studies may contribute to the use of saliva and its components in the diagnosis of lesions and alterations in the oral cavity and in the stomatognathic system as well as in the diagnosis of systemic diseases that change the saliva and its components.

Conclusion

In the investigated sample, the levels of secretory immunoglobulin A (SIgA) and salivary flow did not exhibit a significant difference between smokers and non-smokers.

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