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The Effect of Smoking on Salivary Parameters

Mariana Yordanova[1*], Nikola Shopov[2]

[1]Department of General medicine and Clinical laboratory, Medical University of Varna "Prof. Dr. Paraskev Stoyanov" Varna; Military Medical Academy, Multiprofile Hospital for Active Treatment, Varna, Bulgaria

[2]Military Medical Academy, Centre of Military Medical Expertise Aero and Maritime Medicine, Sofia, Bulgaria

[*]**Corresponding author:** Mariana Yordanova, Department of General medicine and Clinical laboratory, Medical University of Varna "Prof. Dr. Paraskev Stoyanov" Varna ; Military Medical Academy, Multiprofile Hospital for Active Treatment, Varna, Bulgaria. Tel: +359-899599587; Email: m.jordanova.doc@abv.bg

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Abstract

Smoking is a widespread harmful habit and a proven risk factor for a number of inflammatory and malignant diseases of the oral cavity as well as of the respiratory and digestive system.

Aim: The aim of this study is to evaluate and compare the effect of smoking on the salivary composition of whole unstimulated saliva.

Materials and Methods: The study included 186 people, divided into two groups - smokers (77 people) and non-smokers (109) with an average age of 33 (19-57). Ninety-nine of them are men and 87 women. Unstimulated saliva, collected in a passive manner using advanced standard analytical requirements, is used for the analyzes. The following indicators were analyzed: Uric acid (UA), alpha amylase (a-amy), secretory Ig A (sIg A), total protein (TPro), and albumin (alb). Salivary parameters were analyzed with the finished kit of Thermo sciens, Becman Coulter, with the adaptation of the oral fluid methods of the biochemical analyzer Olympus AU 640 and Indiko Plus.

Results: We observed a significant increase in the concentration of salivary total protein, and albumin, and respectively a decrease in UA, amylase and sIg A in smokers, resulting in a change in saliva homeostasis and its antioxidant capacity.

Conclusion: The results show significant quantitative changes in the saliva parameters between smokers and non-smokers, with smoking adversely affecting oral homeostasis and with an increased risk of inflammatory and degenerative changes in the lining.

Keywords: Albumin; Alpha amylase; Saliva; Secretory Ig A; Smoking; Total protein; Uric acid

Introduction

Saliva is the main protector of local soft and hard tissues in oral cavity. It is a liquid, viscous fluid and is a complex system containing 99% water and 1% different low molecular weight substances, enzymes, hormones, antibodies, antimicrobial ingredients and growth factors [1, 2, 3]. Some of these are locally synthesized by the salivary glands, while others are transported from the bloodstream through diffusion processes, active transport or ultrafiltration. Saliva is a mirror of the functional, metabolic, hormonal and emotional state of the body [1].

Approximately 0.5-1.0 liters of saliva are produced daily, with 90% of them being separated from the main salivary glands (parotid, submandibular and sublingual). Saliva has many functions in the oral cavity: it is responsible for digestion, has a protective role in the oral cavity, moisturizes the lining and facilitates the swallowing of food and articulation. It plays an important role in maintaining oral health and oral hygiene, helping to wash pathogenic bacteria and debris. Proteolytic enzymes and antibodies contained in the saliva have antimicrobial activity and are a barrier against the penetration of pathogens into the digestive system. The balance of electrolytes and protein substances in the oral fluid is related to the remineralization of the teeth [2,4].

A number of evidence suggests that smoking is one of the exogenous factors that reduces saliva release and alters

its composition, but the research results are contradictory [5]. Therefore, the purpose of this study is to assess the impact of smoking on salivary laboratory values. Smoking acts mechanically, chemically and thermally on the mucous membrane of the oral cavity, stimulating the salivary glands and resulting in a briefly increased secretion of saliva [5]. Chemical stimulation is explained by the fact that nicotine and cytosine act on nicotine receptors as agonists and increase salivation [5,6]. But in the long run, smoking reduces salivary secretion, the first gland to be affected is parotid. The lost part of its function is compensated by submandibular and sublingual glands that release mucus saliva.

Saliva is the first fluid that cigarette smoke and tar get into direct contact with. They contain over 4,000 different compounds, of which 400 are proven carcinogens - aromatic amines, nitrosamines, oxidants (free oxygen radicals), radioactive elements (Polonium 210) as well as high concentrations of toxic volatile substances. These compounds destroy the protective macromolecules of saliva, enzymes and proteins, thus losing its protective role and the lining becomes susceptible to inflammatory or degenerative changes [7,8]. Smoking induces local oxidative stress and reduces Antioxidant Compounds (AOC), presented by enzyme and non-enzymatic antioxidants. Toxic compounds in tobacco smoke influence the mucosal immune system by disrupting the host's protection, inhibiting granulocyte function [9,10] and cause oxidative stress in tissues [10,11].

Materials and Methods

We examined 186 healthy volunteers, undergoing prophylactic studies, divided into two groups: smokers (77 people) and non-smokers (109) with a mean age of 33 (20-56). The distribution of the study groups is given in Table 1. For the analyzes, unstimulated saliva, collected in a passive manner, using standard pre-analytical requirements, is used. Biological material for each patient is collected in sterile containers between 8 and 10 am after twice rinse with 15.0 ml of distilled water to wash the exfoliated cells. The collected samples (2-3 ml) are placed immediately under refrigeration conditions (2-4°C), then centrifuged for 10 min at 2500 rpm. The supernatant is used for UA, alpha amylase, secretory IgA, total protein and albumin. Biochemical salivary parameters were analyzed with the commercial kits of Thermo sciens, Becman Coulter, Mackerey-Nagel-Germany with the adaptation of the oral fluid methods of the biochemical analyzer Olympus AU 640 and Indiko Plus, and sIg A has been examined by a ready ELISA kit of DiaMetra (Italy).

Values of salivary protein and albumin are too low compared to serum, which requires the use of sensible methods for determining them. To analyze the salivary protein, we apply the dye pyrogolol red, which changes its spectral absorption when bound to the protein. Spectrophotometrically read at 570 λ. This method is sensitive in the low protein concentration range <3.0 g/l. For the determination of albumin, we use an immunological turbidimetric micro albumin test because its salivary values are 100-1000 lower than in serum. Uric acid is enzymatically-colorimetrically determined by the Thermo sciens kit on an Indiko Plus apparatus. A-amylase is analyzed by enzymatic kinetic reactions on the same apparatus due to the higher linear range of the calibration curve, the ability to work with biological saliva material and high dilution at excessive values (especially for amylase). For the calibration purpose, we created a series of matrix calibrators (artificial saliva) with the addition of a certified standard solution.

Statistical Analysis

The differences in the indicator values for smokers and non-smokers were calculated using t -test. The correlations between the different parameters were verified by the Spearman's correlation tests. Differences are considered statistically significant at $p<0.05$. All statistical analyzes were performed using the GraphPad Prism 6 statistical program (Figures 1-6, Table 2).

Groups	Smokers number	Non Smokers number	Age years
Men	42	57	34.45±9.22
Women	35	52	32.12±6.33
Total of Subjects (n 186)	77	109	33.28±7.88 (20-56)

Values are means ± standard deviation.

Table 1: Distribution of age and gender groups.

Results

Of the 186 people surveyed, 41.4% (n 77) were smokers and 58.6% (n 109) were non-smokers. The results of the laboratory tests are presented in Table 2.

Parameters	Unit	Smokers	Non smokers	P values
Uric acid	umol/l	199.3±4.300	231.5± 4.573	p=0.001*
Total protein	mg/l	822.9±36.89	681.8±31.12	p=0.0039*
Albumin	mg/l	57.44±3.780	49.57±1.838	p= 0.0428*
sIg A	mg/l	112.1±5.775	126.6±6.816	NS
a-amylase	U/ml	45.72±6.926	61.11±7.425	NS

Values are means ± standard deviation. Differences were considered statistically significant at $p < 0.05$; NS - not significant

Table 2: Mean values of salivary parameters in both smokers and non-smokers groups.

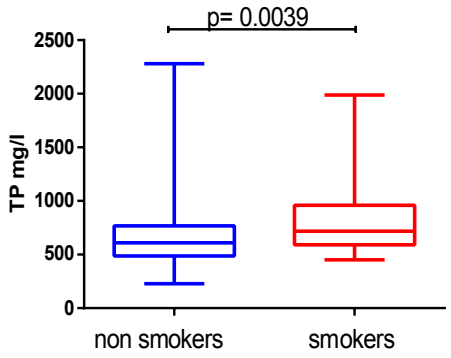

Figure 1: The mean of T protein in the study groups.

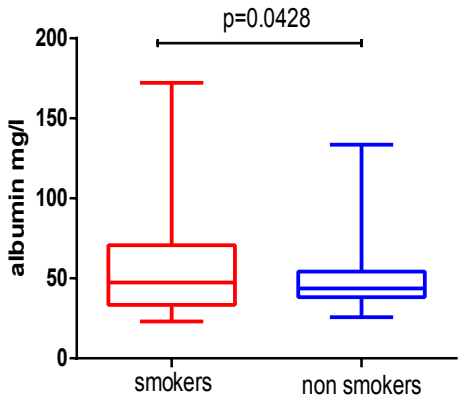

Figure 2: The mean of albumin in the study groups.

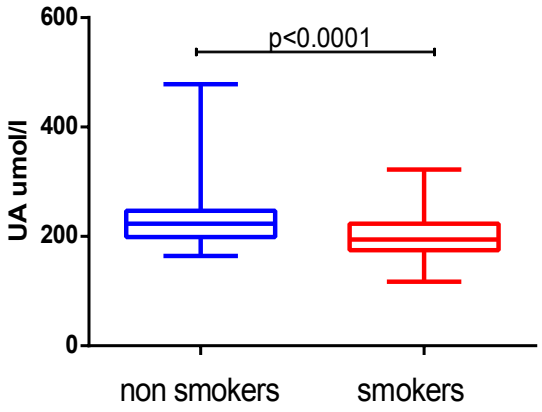

Figure 3: The mean of UA in the study groups.

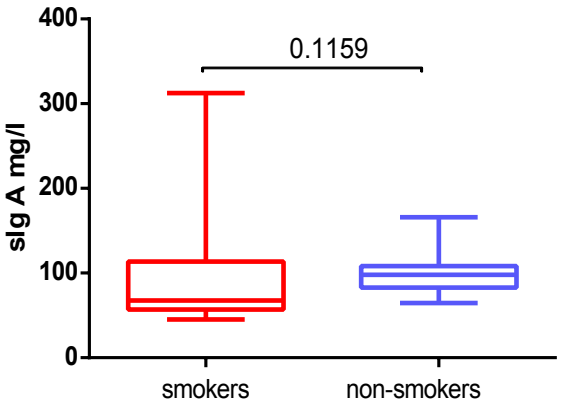

Figure 4: The mean of sIg A in the study groups.

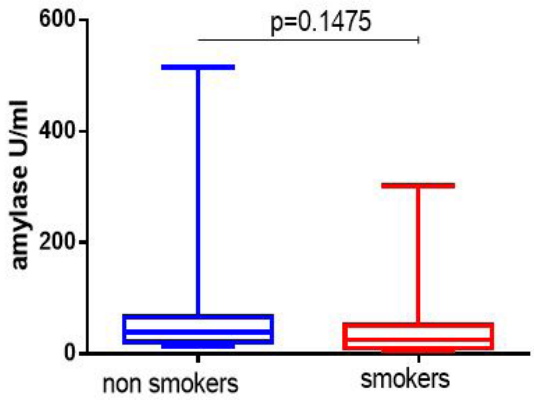

Figure 5: The mean of a-amylase in the study groups.

Discussion

Mucosal protection is accomplished through non-immune mechanisms (integrity of epithelial barrier, commensal microorganisms, secretory activity, etc.) and immune protective factors that are performed by the mucosal immune system (sIg A). Salivary immunoglobulin A is produced by local plasma cells situated in the mucosa and salivary glands and transport protein by ductal epithelial cells [12]. Smoking, acting mechanically and chemically on the mucosa, results in a change in ions, proteins composition, buffer capacity, and pH of the saliva. This is how the balance in the populations of comorbid microorganisms and dysbacteriosis changes.

In smokers, a decrease in secretory immunoglobulin A ($p=0.1159$), although not significantly, compared to the non-smoking control group, is observed. Reduction of sIg A results in suppression of specific immune protection against pathogens microorganisms. This can explain the high percentage of inflammatory diseases of the mucosa and periodontium in smokers. Similar observations have been reported by a number of other researchers [12, 13].

Even in good health, there is a continuous flow and migration of leukocytes from the gingival fluid through the saliva gall. They protect the oral cavity and are an element of the innate immune response. Smokers increase the number of polymorphonuclear neutrophils by impairing T cell immune regulation and B-cell differentiation and maturation. This leads to a decrease in salivary IgA levels. Influencing host protection, inhibiting granulocyte function [10,12] and neutrophil respiratory burst, cigarette smoke causes oxidative stress in tissues [9,11]. Nicotine appears to play an important role in the immune modulation of the host. Our observations are similar with decreasing sIg A in smokers [12, 13, 14].

Common protein is a vital component of saliva and is responsible for most of its functions such as lubrication, physical protection, cleansing, buffering, maintenance of dental integrity, taste and digestion, and antibacterial activity [15, 16]. In general, the main factors influencing the concentration of the whole saliva protein are the velocity of the salivary flow, the protein involvement of the glandular saliva and the crevicular fluid [17, 18]. Tobacco smoking leads to a sustained activation of the sympathetic nervous system, and nicotine causes vasospasm and reduces the blood supply to the salivary glands. As a consequence, decreased salivation and secretion of predominantly small amounts of saliva, rich in proteins, mucins and enzymes are observed. Impaired blood supply, hypoxia and local irritative changes predispose to inflammatory diseases of the oral cavity and periodontium. In our study, we observed significantly higher levels of protein ($p<0.0039$) in smokers compared to non-smokers.

Albumin is the most osmotically active and abundant serum protein that accounts for more than 50% of all plasma proteins. It is synthesized exclusively in the liver and factors that regulate its synthesis are nutrition, hormonal balance and osmotic pressure. Albumin in saliva is considered to be a serum ultra-filtrate which diffuses into oral fluid [15, 18]. It is a protein with antioxidant activity and is an insignificant component of whole saliva, present in all body fluids and tissues have the ability to bind free Oxygen Species (ROS) or inhibit oxidation processes in the cell. In healthy individuals there is a balance between free radicals and antioxidants. Salivary albumin is selectively adsorbed from various oral materials which may allow the attachment of specific bacteria and thus modify the composition of the dental plaque [19, 20]. Salivary protein and albumin are considered as markers for leakage of plasma proteins that arise as a result of an inflammatory process in the oral cavity [20, 21, 22]. An increase in salivary albumin ($p=0.0428$) was observed along with an increase in salivary protein. This difference is statistically significant despite variations in albumin filtration through salivary gill capillaries.

Uric acid is the end product of the purine nucleotides catabolism. It is the most important non-enzymatic antioxidant in the saliva that covers about 85% of the antioxidant capacity. Its concentration correlates well with serum levels. Studies show that its antioxidant function in smokers decreases by more than 1/3 of normal levels [23]. We observed a statistically significant decrease in uric acid in smokers ($p<0.0003$). Initially, as an adaptive mechanism, it can be seen that UA increases to cope with the occurrence of oxidative stress in the oral cavity, which may explain the greater deviation in this group.

Salivary a-amylase is a digestive enzyme with a major role in carbohydrate metabolism. The parotid gland is the main salivary gland, synthesizing and secreting alpha-amylase (70-80% of the total amount of the enzyme). It is assumed that the proportion of amylase in total salivary protein represents from 40 to 50%. The activity of amylase in saliva varies widely between 15,000 and 800,000 U/L due to reverse water absorption in the grooved canal and osmotic transport from the mating canal. For this reason, it is considered a reliable marker for serous cell function [24]. Increasing its values is a typical example of the influence of the sympathetic nervous system (stress or beta mimetic stimulation) on salivation [25]. But this effect is exhausted by the duration of smoking and the number of daily cigarettes. Our data show decreased amylase activity in smokers, although we did not take into account the duration of smoking or the number of smoked cigarettes. On the other hand, long-term eating habits (increased consumption of starchy foods) can also affect enzyme levels [26, 27]. The large variation in amylase levels in the non-smoker group can be explained by the diet or mental state. Part of this group are students after passing the exam. A number of studies have shown a correlation between changes in salivary a-amylase activity and gastric events. Amylase has not only digestive function but also has antibacterial action. It is believed to be able to modulate the adhesion of bacteria to the surface of the lining. It protects the stomach from microbial attack [28].

Conclusion

Saliva is a noninvasive and accessible bio fluid that permits early detection of oral and systemic diseases. Today salivary diagnostics is a promising tool for diagnostic processes and clinical monitoring. Saliva is a protector providing immune and antioxidant protection to the oral cavity and digestive system, which can be destroyed by toxic and carcinogenic substances contained in cigarette smoke. The results of our study found significant quantitative changes in saliva parameters between smokers and non-smokers, with smoking adversely affecting oral homeostasis and increasing the risk of inflammatory and degenerative changes in the lining.

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