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THE CONNECTION BETWEEN ORAL HEALTH AND SALIVARY LEVELS OF TOTAL PROTEINS, CALCIUM AND PHOSPHATES

ПОВРЗАНОСТ НА ОРАЛНОТО ЗДРАВЈЕ И САЛИВАРНИТЕ НИВОА НА ВКУПНИТЕ ПРОТЕИНИ, КАЛЦИУМОТ И ФОСФАТИТЕ

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Abstract

Aim of the study: To determine the association between oral hygiene and the values of total protein, calcium. and phosphate in the saliva in young, healthy population. **Material and methods:** The research was conducted on 41 respondents of both sexes, aged 19-24 without pathological changes in the oral cavity. Unstimulated saliva was collected using the spitting method into graduated test tubes for 5 min. The samples were then centrifuged at 4000 rpm for 20 minutes. The level of protein in the samples was determined using the Biuret method, the concentration of Ca was determined with Calcium-cresolphthalein, and phosphate concentration was determined with phosphomolybdate/UV. The concentrations were determined spectrophotometrically with UltroSpec 1000. The index of dental plaque, gingival inflammation and dental calculus were clinically noted, indicating the level of oral hygiene in each individual. The obtained data from the examinations were statistically processed using the Pearson's correlation coefficient. **Results:** We detected a weak correlation between the index values of the dental plaque and total salivary protein p=0.000 and p=0.003, respectively. Weak positive correlation was noted between index values of dental calculus and salivary calcium p=0.000, and of calculus and salivary phosphate p=0.015. **Conclusion:** The results showed that there is a connection between gingival inflammation, dental calculus and values of salivary protein, calcium, and phosphate in the saliva of this group of respondents. **Keywords:** saliva, salivary proteins, calcium, phosphates, oral health.

Апстракт

Цел: да ја утврдиме поврзаноста помеѓу оралното здравје (хигиена) и вредностите на вкупните протеини, калциумот и фосфатите во плунката кај млада, здрава популација. **Материјал и метод:** спроведено е испитување на 41 испитаник од двата пола, на возраст од 19 до 24 год. без патолошки промени во оралната празнина. Колекционирана е нестимулирана плунка со методот на исплукување во градуирана епрувета во тек на 5 мин. Примероците беа центрифугирани на 4000 вртежи во тек на 20 минути. Нивото на вкупните протеини во примероците беше одредувано со помош на Модифицирана Биуретска метода. Концентрацијата на Са во плунката ја одредувавме со Calcium-cresolphthalein. Саливарните фосфати ги определувавме со phosphomolybdate/ UV. Концентрациите беа одредени со спектрофотометарот UltroSpec 1000. Клинички ги нотиравме индексот на дентален плак, гингивална инфламација и забен камен, кои укажуваат на степенот на орална хигиена кај секоја индивидуа. Добиените податоци од лабораториските и клиничките испитувања беа статистички обработени, беше користен Pearson-овиот коефициент на корелација. **Резултати:** Утврдивме позитивна корелација помеѓу индексните вредности на ГИ и денталниот плак со вкупните саливарни протеини за p=.000 и p=.003 соодветно. Слаба позитивна корелација нотиравме помеѓу индексните вредности на калкулусот и вкупниот саливарен калциум за p=0.000, како и на калкулусот со саливарните фосфати p=0,015. **Заклучок:** резултатите покажаа дека постои поврзаност помеѓу гингивалната инфламација и денталниот калкулус и вредностите на саливарните протеини, калциумот и фосфатите во плунката кај оваа група испитаници. **Клучни зборови:** салива, саливарни протеини, калциум, фосфати, орална хигиена.

Introduction

The oral cavity is a specific ecosystem with many functions, where saliva as a biological fluid plays a major role in maintaining oral homeostasis and oral health. Saliva, as a secretory product of the large and small salivary glands, modulates this ecosystem, primarily through constant rinsing of the teeth and oral mucosa, protection against microorganisms, participation in mastication, taste perception, prevention of oral infections and dental caries[1,2,3]. Saliva consists of organic, inorganic material and macromolecules. Inorganic substances, such as calcium and phosphate ions, are involved in the formation of the dental tissues, dental calculus, but also in the protection against caries. Salivary proteins have a protective, antimicrobial and lubricating function (mucin) that provides a

barrier between toxins and soft oral tissues. Salivary proteins modulate the activity of calcium and phosphate ions. Saliva is an ionic reservoir of calcium and phosphates necessary for mineralization of initial carious lesions[4]. Through constant rinsing of the oral cavity, saliva plays a huge role in maintaining oral hygiene and oral health.

This study aims to determine the connection between oral health (oral hygiene) and salivary levels of total proteins, calcium, and phosphate in young healthy population.

Material and methods

The research was conducted with 41 young healthy respondents of both sexes, aged 19-24, students at the Faculty of Dental Medicine within UKIM, without pathological changes in the oral cavity. The study included those without systemic diseases and those who had not used certain medications in the last three months. Unstimulated saliva was collected from all respondents using the spitting method according to the recommendations proposed by Navazesh[5]. The subjects were spitting in polypropylene, graduated test tubes for 5 min. The tubes were placed in an ice-filled glass container to prevent protein breakdown. Students were advised not to consume food and drinks for at least 90 minutes before saliva collection. Before collecting, they first thoroughly rinsed their mouths with deionized water, then they were comfortably placed in a sitting position with their head slightly tilted forward and proceeded to spit. The collected saliva samples were briefly vortexed in a Vortex apparatus for about 1 min and then centrifuged at 4000 rpm for 20 minutes.

The level of total protein in the samples was determined using the Modified Biuret method, by measuring the absorbance of the test sample. The concentration of Ca in saliva was determined with Calcium-cresolphthalein. The principle is based on the reaction of the calcium from the analytical sample with o-cresolphthalein from the Biuret solution forming a red colored-complex that is measured spectrophotometrically[6].

Salivary phosphates were determined with phosphomolybdate/UV because inorganic phosphates from the saliva samples react with molybdate from the solution in acidic pH, thus forming phosphomolybdate-complex, which can be measured spectrophotometrically. The concentrations were determined with the UltroSpec 1000 spectrophotometer apparatus.

The index of dental plaque, gingival inflammation and dental calculus were clinically noted, indicating the level of oral hygiene in each individual. The obtained data from the laboratory and clinical examinations were statistically processed using Pearson's correlation coefficient.

Results

41 respondents participated in the study, 12 (29.27%) of the respondents were male, while 29 (70.73%) were female. Regarding the representation of gender, we registered a statistically significant difference of $p<0.05$ between the representation of males in relation to females (Table 1).

Table 1. Representation of the respondents in relation to gender

	n	Percent %
female	29	70.73171
male	12	29.26829

Table 2. Average age of respondents included in the study

	Average age	Min.	Max.	Std.Dev.
Age	19.95122	19.00000	24.00000	0.835201

Table 4. Distribution of the respondents according to smoking habits

	n	%
Non - smokers	26	61.90476
Smokers	15	35.71429

Table 3. Average values of the examined salivary components

	average	min.	max.	Std.Dev.
Amount of saliva excreted (ml/min)	0.358537	0.150000	0.700000	0.150276
Proteins (mmol/L)	1.902927	0.100000	3.960000	0.922392
Calcium (mmol/L)	1.092439	0.210000	6.710000	1.035939
Phosphates (mmol/L)	3.110976	1.250000	6.440000	1.241102

Table 5. Distribution of respondents according to the values of the indices of oral hygiene and gingival inflammation

	Dental calculus		Gingival inflammation		Dental plaque (biofilm)	
Index values	n	Percent %	n	Percent %	n	Percent %
0	9	21.95122	4	9.75610	/	/
1	15	36.58537	14	34.14634	22	53.65854
2	13	31.70732	18	43.90244	17	41.46341
3	4	9.75610	5	12.19512	2	4.87805

Table 6. Average values of total salivary proteins in smokers and non-smokers

mmol/L	Non-smokers	Smokers	t-value	df	p
Proteins	2.144231	1.484667	2.323465	39	0.025459

Table 7. Difference between average values of total salivary proteins in males and females

mmol/L	female	male	t-value	df	p
Proteins	1.942069	1.808333	0.418025	39	0.678221

Table 8. Difference between average salivary calcium values in smokers and non-smokers

	Non-smokers	Smokers	t-value	df	p
Calcium	0.832308	1.543333	-2.21817	39	0.032436

The average age of the respondents was 19.95 ± 0.83. The youngest respondent was 19 years old, and the oldest respondent was 24 years old. (Table 2)

In our study, most of the respondents 26 (61.9%) were non-smokers and 15 (35.7%) were smokers. (Table 4)

The obtained results regarding the presence of dental calculus showed that in 9 respondents was noted index 0 (21,95%), in 15 was noted index 1 (36,58%), in 13 respondents was present index 2 (31,70%), and in 4 subjects was observed index 3 (9,75).

Regarding the presence of gingival inflammation, the results showed that in most of the participants, 18 (43,90%) was present index 2, and in 14 (34,14) index 1 of gingival inflammation. In only 4 subjects (9,75%) we did not register inflammatory changes in the gingiva, and in 5 (12,2%) we noted index 3 of gingival inflammation.

Regarding the obtained results for presence of dental plaque, we noticed that none of the participants in the study had an index 0, in 22 (53,65%) subjects we registered index 1, in 17 index 2 (41,46%), and only in 2 participants (4,88)- index 3 dental plaque. (Table 5.)

We registered a statistically significant difference for p=0.025459 between the average values of total salivary proteins in smokers and non-smokers. (Table 6.)

We did not register a statistically significant difference (p=0.678221) between the average values of total salivary proteins between male and female respondents. (Table 7.)

We registered a statistically significant difference for p=0.032436 between the average values of calcium in smokers and non-smokers. (Table 8.)

Table 9. Difference between average salivary calcium values in males and females

	male	female	t-value	df	p
Calcium	0.89069	1.58000	-2.01097	39	0.051278

Table 10. Difference between average salivary phosphate values in non-smokers and smokers

	Non-smokers	Smokers	t-value	df	p
Phosphates	2.906923	3.464667	-1.40268	39	0.168624

Table 11. Difference between average salivary phosphate values in males and females

	male	female	t-value	df	p
Phosphates	3.030345	3.305833	-0.641916	39	0.524683

There was no statistically significant difference between average salivary calcium values in males and females (p= 0,051) (Table 9.)

We did not observe a statistically significant difference for p = 0.168 between the average salivary values of phosphates in smokers and non-smokers. (Table 10.)

We have not determined a statistically significant difference between the average values of phosphates in saliva in male and female respondents. (Table 11.)

Table 12. Correlation between index values of examined clinical parameters and total salivary proteins

	Total proteins
Dental calculus	r = - 0.1873
	p = 0.241
Gingival inflammation	r = 0.7691
	p =.000
Dental plaque (biofilm)	r = 0.4482
	p =.003

The results show presence of positive correlation between total salivary proteins and index of gingival inflammation (r= 0,76 p =0.000), and presence of weak positive correlation between index of dental plaque and salivary proteins (r =0,44; p =0,003). (Table 12.)

The Pearson correlation index indicates that there is a positive correlation between salivary calcium and index of dental calculus (r =0,53 p =0,000). (Table 13.)

Table 13. Correlation between index values of clinical parameters and salivary calcium

	Calcium
Dental calculus	r = 0.5258
	p = 0.000
Gingival inflammation	r = 0.2333
	p = 0.142
Dental plaque (biofilm)	r = 0.2386
	p = 0.133

Table 14. Correlation between index values of clinical parameters and salivary calcium

	phosphates
Dental calculus	r = 0.3763
	p = 0.015
Gingival inflammation	r = 0.1893
	p = 0.236
Dental plaque	r = 0.1333
	p =.406

We found the presence of a positive correlation between dental calculus index and salivary phosphates (r=0,36; p=0,015) (Table 14.)

Discussion

The average amount of saliva excreted in our subjects is 0.358 ml /min, which is within the normal values of salivation in most of the population 0.33-1.42ml/min[7]. The results showed that there were no significant differences in the amount of unstimulated saliva between smokers and non-smokers. We believe that this is due to the age of the respondents, young individuals, short "experience" of smoking and preserved function of the salivary glands, as well as the small number of smokers who participated in the study – 15 of the subjects are smokers[8].

Regarding the total salivary proteins, we noticed a significant difference between smokers and the group of non-smokers for p = 0.02, i.e. higher protein concentration in the saliva of non-smokers 2.144 mmol /L. In terms of gender, there was no significant difference between the two groups of respondents (p = 0.678221). (Table 6)

The concentration of the various components of saliva is significantly affected by variations in saliva secretion. Hormonal factors, external influences and systemic diseases have a significant influence on these variations in the composition of saliva. Literature data indicate that smokers have a change in saliva quality. Most of them have thick, sticky saliva. It has been proven that due to the harmful effects of nicotine, the parotid salivary glands that secrete serous saliva suffer first, and the loss of their function is compensated by the submandibular and sublingual salivary glands that secrete predominantly sero-mucous and mucous secretions. This explains the presence of thick saliva in smokers. Harmful substances from cigarette smoke destroy the protective macromolecules of saliva, enzymes, and proteins, therefore it loses its protective role[9,10].

The results showed that there is a significant difference in the presence of calcium in the saliva between smokers and non-smokers (p=0.032), i.e. more Ca was found in the saliva of smokers (1,543 mmol / l) (tab.8). We did not determine a significant difference in the presence of Ca in the saliva of the respondents in terms of gender (p=0,051) (Table 9).

Based on the obtained results, we did not find significant differences in the average values of phosphates in saliva, neither between the group of smokers and non-smokers (p=0.168), nor in terms of gender (p =0.524683). (tab. 10 and tab.11)

We found a positive correlation between the level of total protein in the saliva of our subjects and gingival inflammation r=0.76 (p=0.000), as well as a positive correlation between total protein and dental plaque r=0.44 (p=0.003) (Table 12). We believe that the presence of gingival inflammation causes an increase in total protein in saliva. Gingival inflammation causes vasodilation and increased transudation of fluid and proteins from the blood vessels of the gingiva into the gingival fluid, thus increasing the amount of protein in the saliva. Some studies suggest that total salivary protein levels increase in individuals with gingivitis and periodontitis[11]. The main factors influencing the concentration of protein and the protein composition of the saliva are salivary flow, the presence of proteins originating from the salivary glands themselves, as well as those originating from the gingival fluid. The increased protein levels may be due to local synthesis and secretion by the glands themselves. Additionally, glandular-derived proteins, Cystatin C as well as amylase, which are significantly increased in patients with periodontitis, confirm the glandular origin of these proteins[12].

The increase of salivary albumin also plays an important role in the increase of total proteins. Albumin is detected as a minor component in whole saliva (parotid, submandibular and sublingual), but a significant increase in its concentration has been observed in patients with gingivitis and periodontitis[11]. Changes in the concentration of endogenous immuno-reactive proteins such as cystatin and secretory IgA, as well as increased gingival fluid secretion, have been reported in these patients[12,13]. This may indicate that gingival bleeding, and the amounts of GCF derived proteins or immuno-reactive proteins may be one of the causes of oral malodor, because of increased levels of sulfur components[14]. It was reported that the decrease in salivary flow could also result in increased protein concentration. Lower flow rate of saliva secretion could affect oral conditions by leading to increased accumulation of dental plaque and bacteria[15] which can affect oral health.

Gingival fluid is a physiological fluid, but also an inflammatory exudate originating from the blood vessels of the gingival plexus in the gingival corium. As it traverses through the inflamed periodontal tissues to the sulcus, important biological molecular markers are gathered from the surrounding tissues and dissolved into the saliva[16].

The hypothesis that periodontal pathogens induce inflammatory responses resulting in increased levels of salivary albumin and total protein is well known. P. gingivalis, P. intermedia and T. denticola are predominantly present in patients with periodontitis. T. denticola increased the levels of salivary albumin and total proteins as the proteins existing in the periodontal pocket, including immunoglobulins and albumin as potential energy sources for T. denticola. Hollman and Van Der Hoeven[18] have reported that degradation of albumin by T. denticola was not detected but suggested that T. denticola occurs in close association with strong proteolytic bacteria such as P. gingivalis and T forsythia in the subgingival plaque. Thus, controlling the microbes in turn decreases the

inflammatory response, which in turn decreases the plasma leakage in the saliva through GCF.

The presence of Ca, phosphates, and other inorganic ions, especially fluoride in saliva, provides an important protective role and maintains the integrity of dental tissues. The medium rich in Ca and phosphates allows the mineralization of the initial carious lesions and the demineralized areas of the enamel. The balance between demineralization and mineralization depends on the saturation of saliva with calcium and phosphates, saliva flow, its pH, stimulated or unstimulated saliva, and the level of salivary alkaline phosphatase. Salvolini[19] studied 100 healthy subjects of both genders, aged 10-80 years, and suggested that the concentrations of calcium and phosphorus were not affected by age.

The average values of the concentration of calcium in saliva in physiological conditions at healthy individuals is 8.8 - 10.5 mg/dL, but these values vary in stimulated and unstimulated saliva. Gauri, Nagarajappa et al.[20] in their studies found a low mean Ca value of 5.87 mg/dL in unstimulated saliva, and after stimulation, the Ca concentration values increased to 7.17 mg /dL. Jarvinen[21,22] describes similar findings in his study, low values of calcium and phosphate concentrations in unstimulated saliva, which he explains with the presence of a small amount of unstimulated saliva.

Vogel[23] indicates that unstimulated saliva is well-saturated with phosphates, but a rapid decrease in their concentration is observed during stimulated secretion (eg. chewing gum).

In our study, we found the presence of a moderate positive correlation between Ca in saliva in our subjects and dental calculus r=0.52 (p=0.000). (Table 13). We also found presence of a weak positive correlation between salivary phosphates and dental calculus in our subjects r=0.37 (p=0.015) (tab 14).

This means that increased concentrations of Ca and phosphates in saliva lead to increased presence of dental calculus. We believe that the saturation of saliva with these ions causes apposition of these minerals in the present dental biofilm, especially in patients who do not maintain proper oral hygiene, which leads to calculus formation. Mineralization can begin as early as 24-48 hours. Calcification starts in separate foci on the inner surface of plaque, these foci of mineralization gradually increase in size and coalesce to form a solid mass of calculus[24]. It is a well-known fact that dental calculus itself is not an including agent for pathological changes that occur in gingival tissues; instead, it is covered with a layer of unmineralized plaque which is proven to be the key etiological agent involved in these pathogenic mechanisms. Dental calculus plays an instrumental role in further deterioration of the periodontal disease and oral health disorders. On the other hand, reduced concentration of calcium ions can cause demineralization and carious lesions of the hard tooth structures and the possibility of accumulation of dental plaque in those places[24].

Phosphorus is the second most important mineral in the human body after calcium. About 80%-90% of phosphorus is present in the bones and teeth in the form of hydroxyapatite and the rest is present in the extracellular fluid (ECF), soft tissue and erythrocytes. In living tissue, phosphorus exists in the form of phosphate (PO4-3). According to the theory of mineral precipitation on the formation of calculus, calcification will occur when the pH, calcium, and phosphate saliva concentrations are high enough to cause precipitate calcium phosphate salts[25]. Prashaanthi N. et al in their study report that patients with periodontitis have higher level of calcium and phosphates in saliva than healthy subjects[25]. Other authors[26] in their study report that supersaturation of saliva with respect to calcium phosphate salts is the driving force of calculus formation. They found a positive correlation between the mean values of salivary calcium concentration with the amount of calculus formation. However, the increased concentration of phosphate ions and the presence of bicarbonate in the stimulated saliva have an influence on the regulation of acid-base balance and may stimulate the process of mineralization of tooth structures.

Conclusion

The results of this study show that there is a connection between gingival inflammation, dental calculus and the values of salivary proteins, calcium, and phosphate in saliva in this group of respondents. Saliva is the most available and non-invasive bio-fluid of the human body, which permanently rinses the oral cavity while trying to cope with an ever-changing milieu. Saliva is critical to the preservation and maintenance of oral health, and any changes in its amount or quality may alter the oral health status.

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