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SALIVARY CALCIUM AND PHOSPHATE LEVELS IN CORRELATION WITH DENTAL CARIES INTENSITY

КОНЦЕНТРАЦИЈА НА САЛИВАРНИ КАЛЦИУМ И ФОСФАТИ ВО КОРЕЛАЦИЈА СО ИНТЕНЗИТЕТОТ НА ДЕНТАЛЕН КАРИЕС

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Abstract

Objective: The objective of our study was to determine and compare calcium and phosphate levels in unstimulated saliva in patients with varying caries intensity. **Material and methods:** The study involved 109 subjects from both sexes, from 12 to 15 years of age, divided into 3 groups according to their caries intensity: group with very low and low caries intensity, involving 31 (28.44%) subjects; group with moderate caries intensity involving 30 (27.52%) subjects; and a group with high and very high caries intensity, involving 48 (44.04%) subjects. Saliva samples were collected with the spitting method, according to the recommendations by Navazesh. Salivary calcium and phosphate levels were determined using the spectrophotometric methods CALCIUM ARSENAZO and PHOSPHOMOLYBDATE/UV accordingly. **Results:** The average concentration of salivary phosphates was 7.86 ± 3.3 mmol/L in subjects with very low and low caries intensity, 5.96±2.9 mmol/L in subjects with moderate caries intensity, and 5.08 ± 2.6 mmol/L in subjects with high and very high caries intensity. Post-hoc analysis for inter-group comparisons confirmed the statistically significant difference between groups with very low and low caries intensity and those with high and very high caries intensity (p=0.0005). The statistically significant difference was observed in calcium concentration as per caries intensity (p<0.0001). Subjects from the group with high and very high caries intensity showed a significantly lower level of calcium compared to the subjects from the group with very low and low caries intensity (p=0.000001), and a significantly lower level of calcium compared to the subjects with moderate caries intensity (p=0.0014). There is a correlation between the salivary calcium and phosphate levels and caries intensity (R=0.353, R=0.51 respectively). **Conclusion:** Salivary calcium and phosphate levels were significantly lower in the subject group with high and very high caries intensity, compared to the subject group with very low and low caries intensity. The intensity of caries significantly correlates with the concentration of salivary phosphates and salivary calcium. **Key words:** Caries intensity, saliva, salivary calcium, saliva phosphates.

Апстракт

Цел на трудот: Целта на нашето истражување беше да се утврди ис пореди нивото на калциум и фосфати во нестимулирана плунка кај пациенти со различен интензитет на кариес. **Материјал и методи:** Истражувањето вклучи 109 испитаници од двата пола, на возраст од 12 до 15 години, поделени во 3 групи според интензитетот на кариес: група со многу низок и низок интензитет на кариес, вклучувајќи 31 испитаник (28.44%), група со среден интензитет на кариес, вклучувајќи 30 испитаници (27.52%) и група си висок и многу висок интензитет на кариес, вклучувајќи 48 испитаници (44.04%). Примероците од плунка беа колекционирани со методот на исплукување, според порепораките на Navazesh. Нивоата на саливарен калциум и саливарни фосфати беа определени со спектрофотометриските методи CALCIUM ARSENAZO и PHOSPHOMOLYBDATE/UV соодветно. **Резултати:** Просечната концентрација на саливарните фосфати беше 7.86 ± 3.3 mmol/L кај испитаниците со многу низок и низок интензитет на кариес, 5.96±2.9 mmol/L кај испитаниците со среден интензитет на кариес, и 5.08 ± 2.6 mmol/L кај испитаниците со висок и многу висок интензитет на кариес. Post-hoc анализата за интергрупна споредба потврди статистички значајна разлика помеѓу групите со многу низок и низок интензитет на кариес и оние со висок и многу висок интензитет на кариес (p=0.0005). Статистички сигнификантната разлика беше потврдена и за концентрациите на калциум во однос на интензитетот на кариес (p<0.0001). Испитаниците од групата со висок и многу висок интензитет на кариес презентираше сигнификантно пониски нивоа на калциум во споредба со испитаниците од групата со многу низок и низок интензитет на кариес (p=0.000001), и сигнификантно пониски нивоа на калциум во споредба со испитаниците од групата со среден интензитет на кариес (p=0.0014). Постои корелација помеѓу нивоата на саливарен калциум и фосфати и интензитетот на кариес (R=0.353, R=0.51 соодветно). **Заклучок:** Нивоата на саливарен калциум и саливарни фосфати беа сигнификантно пониски кај испитуваната група со висок и многу висок интензитет на кариес, во споредба со испитуваната група со многу низок и низок интензитет на кариес. Интензитетот на кариес сигнификантно корелира со концентрацијата на саливарните фосфати и саливарниот калциум. **Клучни зборови:** Интензитет на кариес, плунка, саливарен калциум, саливарни фосфати.

Introduction

Dental caries is considered the most common disease in people, following the common flu, and is without a doubt a public health problem which is among the most widely spread global diseases connected with dental biofilm[1]. It is a multi-factor chronic disease of the hard dental tissues, characterized with demineralization of the non-organic part and destruction of the organic content of teeth. The World Health Organization (WHO) has defined caries as a localized post-eruptive process of external origin, which involves softening of the hard dental tissue and consequently creation of a cavity[2].

The risk of occurrence of caries includes physical, biological, ecological factors, factors connected to behavior and lifestyle, cultural and hygiene-dietary habits[3,4].

Effective preventive measures against occurrence of caries include inhibiting cariogenic microorganisms, mechanical control of the dental plaque and controlled intake of sugars, in order to reduce the amount of biofilm and the levels of specific pathogens.5 The World Health Organization (WHO) has reported that 60% to 90% of school children and almost 100% of adults worldwide suffer from caries. Therefore, prevention against caries plays an indispensable role in the promotion of public health[6].

Many authors have emphasized the importance of the relation between dental biofilm and dietary sugar, as the primary etiological factors for the incidence of dental caries, whereas, if one of these factors is absent, caries cannot occur[7-10].

Literature presents convincing reasons for using saliva as a diagnostic fluid. As a clinical tool, it offers numerous advantages compared to serum, mainly because it is easy to collect, store and deliver in sufficient amounts for further analysis[11]. Due to the non-invasiveness of the saliva collection procedure, patients are less anxious and uncomfortable, which makes it easy to repeat the sampling and monitor the disease over time[12]. Saliva is also easier to handle during diagnostic procedures because it does not clot, reducing the need of additional manipulation. It greatly affects initiation, maturing and metabolism of the dental plaques[13].

Calcium and trivalent phosphate ions play an important role in the protection of teeth against development of caries and erosions which, together with hydroxyl ions, maintain the mineral saturation of the saliva compared to the minerals in the dental tissues, a fact that is highlighted by numerous authors[14-22]. The structure of the dental enamel is predominantly composed of hydroxyapatite which involves ions of calcium and phosphates[15]. The high concentrations of calcium and phosphates in the saliva enable ionic exchange with the surface of the dental tissues, which starts with eruption of teeth and lasts until their maturity. This provides for remineralization of the initial carious process, before tooth cavity occurs, mainly due to the presence of ions of calcium and phosphates in the saliva[23].

The demineralization of hard dental tissues occurs when there is a non-proportional content of calcium and phosphate minerals between the tooth and the oral environment. In such cases, hydroxyapatite crystals of the enamel dissolve as a result of the acids produced by microorganisms, which leads to demineralization of the tooth. The low level of calcium and phosphates in the saliva affects the balance between the process of demineralization and remineralization, and the results in dental caries. This also explains the importance of salivary concentrations of calcium and phosphate ions in the maintenance and preservation of tooth integrity against the process of demineralization[24].

Since literature data indicate a connection between the concentration of salivary calcium and salivary phosphates and carious processes, the objective of our research was to determine and compare calcium and phosphate levels in unstimulated saliva in patients with varying caries intensity.

Material and method

This study included 109 subjects from both sexes, from 12 to 15 years of age, with maintained general and oral health. The subjects were:

- pupils in the 6th and 7th grade from the primary schools „Petar Pop Arsov“ and „Dimitar Pop-Georgiev Berovski“,
- pupils in the first year of the Medical high school „Dr.Panche Karagjozov“ and
- patients from the PHI University Dental Clinical Center „St. Panteleimon“ - Clinic for Pediatric and Preventive Dentistry and the Clinic for Orthodontics.

We obtained permits from the school authorities, as well as written consent from the parents for the implementation of the research.

The criteria for inclusion of the subjects were as follows:

- Children with permanent dentition, from 12 to 15 years of age (we avoided mixed dentition because caries in primary teeth may compromise the results),
- Children without localized or systemic disease affecting saliva secretion,
- Permanent residents of the city of Skopje who regularly consume local water.

Children who couldn't cooperate during the examination and collection of material were excluded from this study.

The subjects were divided in three groups according to the caries intensity, i.e. according to the DMFT index values (WHO, Geneve, 2000) in the following manner:

- Subjects with DMFT index values between 0.0-2.4 were categorized in the group with very low and low caries intensity
- Subjects with DMFT index values between 2.5-3.8 were categorized in the group with moderate caries intensity, and
- Subjects with DMFT index values between 3.9 and over 5.6 were categorized in the group with high and very high caries intensity.

All subjects underwent clinical, laboratory examinations, survey and statistical analysis of the obtained results.

Clinical trials

The clinical research was conducted in the aforementioned institutions using a probe and dental mirror, and we have determined the DMFT index value using the Klein-Palmer system for every subject.

Establishment of the DMFT index, using Klein-Palmer system[25], was achieved by summarizing the total number of decayed, extracted and restored-filled permanent teeth.

DMFT = Decayed teeth + missing due to caries + filled teeth

Saliva sample collection, processing and laboratory analyses:

The saliva sample collection, processing and laboratory analyses were conducted in the Biochemistry laboratory at the Ss. Cyril and Methodius University in Skopje, Faculty of Dentistry Skopje, Department of Oral and Periodontal Diseases. According to the recommendations by Navazesh[26], unstimulated saliva from all subjects was being collected for 10 minutes. Subjects were instructed not to eat or drink liquids except water for 90 minutes before saliva collection.

The collected saliva was first mixed using a vortex device (DRAGONLAB MX-S), at highest speed-2, after which, it was centrifuged for 10 minutes, at 4000 RCF (xg) in centrifuge (BIOBASE – High Speed Refrigerated Centrifuge). After centrifuging, we collected 500-1000µl of the supernatant of the centrifuged saliva, using a micropipette, and distributed the samples into small plastic single use tubes (Eppendorf tubes). The processed saliva samples were frozen at -20^{O}C, and the analysis of samples was conducted 15 days as of the date of freezing at the latest.

The next phase of our study was to determine the salivary levels of calcium and phosphates. The analyses were made upon fast defrosting of the samples.

Salivary calcium and phosphate levels were determined spectrophotometrically using BioSystem reagents, with the following methods accordingly: CALCIUM ARSENAZO (650 nm) and PHOSPHOMOLYBDATE/UV (340nm).

All the materials and reagents we used had the relevant degree of purity necessary for analysis.

The data were statistically processed using Statistica SPSS v23.0 for Windows.

Results

This study included 109 subjects distributed in 3 groups according to caries intensity: a group with very low and low caries intensity, involving 31 (28.44%) subjects; a group with moderate caries intensity, involving 30 (27.52%) subjects; and a group with high and very high caries intensity, involving 48 (44.94%) subjects. (table 1)

Table 1. Distribution of samples according to caries intensity

Caries intensity	n (%)
P	31 (28.44)
Y	30 (27.52)
A	48 (44.04)

p (group with very low and low caries intensity)
y (group with moderate caries intensity)
a (group with high and very high caries intensity)

Table 2 shows the descriptive parameters of the analyzed salivary biomarkers (phosphates and calcium).

The average concentration of salivary phosphate levels was 7.86 ± 3.3 mmol/L, 5.96 ± 2.9 mmol/L and 5.08 ± 2.6 mmol/L in the subject group with very low and low caries intensity, the group with moderate intensity, and the group with high and very high caries intensity, respectively. The median value of the salivary levels was 7.98 mmol/L, 5.96 mmol/L and 4.3 mmol/L in the subject group with very low and low caries intensity, the group with moderate intensity, and the group with high and very high caries intensity, respectively (table 3).

Salivary calcium showed average and median values of 1.37 ± 0.3 µmol/L and 1.47 µmol/L in the group with

Table 2. Statistical parameters of salivary markers

Parameter	Descriptive statistics		
	mean ± SD	min - max	median (IQR)
Phosphates (mmol/L)	6.11 ± 3.1	1.08 – 14.05	5.22 (3.74 – 7.98)
Calcium (mmol/L)	1.22 ± 0.4	0.64 – 3.46	1.21 (0.98 – 1.42)

Table 3. Values of saliva phosphates – groups according to caries intensity

Groups	Descriptive statistics - **Phosphates (mmol/L)**		p-value
	mean ± SD	**median (IQR)**	
p	7.86 ± 3.3	7.98 (4.95 – 10.88)	H=14.0 ***p=0.0009 post-hoc p vs a ***p=0.0005
y	5.96 ± 2.9	5.96 (3.53 – 7.35)	
a	5.08 ± 2.6	4.3 (3.405 – 5.675)	

p (group with very low and low caries intensity)
y (group with moderate caries intensity)
a (group with high and very high caries intensity)
H (Kruskal-Wallis test), post-hoc (Mann-Whitney test); ***p<0.0001

Table 4. Values of salivary calcium – groups according to caries intensity

Groups	Descriptive statistics - **Calcium (mmol/L)**		p-value
	mean ± SD	**median (IQR)**	
p	1.37 ± 0.3	1.47 (1.15 – 1.52)	H=28.6 ***p=0.00000 post-hoc p vs a ***p=0.000001 y vs a **p=0.0014
y	1.28 ± 0.2	1.355 (1.21 – 1.41)	
a	1.09 ± 0.4	1.055 (0.89 – 1.14)	

p (group with very low and low caries intensity)
y (group with moderate caries intensity)
a(group with high and very high caries intensity)
H(Kruskal-Wallis test), post-hoc (Mann-Whitney test); ***p<0.0001

Table 5. Values of salivary calcium – groups according to caries intensity

Correlation			
Caries intensity	**Spearman R**	t	p-value
&Phosphates (mmol/L)	0.353	3.898	***0.00017
&Calcium (mmol/L)	0.510	6.136	***0.00000

*p<0.05, ***p<0.0001

very low and low caries intensity, respectively, 1.28 ± 0.2 µmol/L, and 1.355 µmol/L in the group with moderate caries intensity, respectively, 1.09 ± 0.4µmol/L, and 1.055 µmol/L in the group with high and very high caries intensity, respectively (table 4).

Table 5 shows the correlation between salivary biomarkers and caries intensity. The analysis showed that the intensity of caries significantly correlates with the concentration of salivary phosphates (p=0.00017) and salivary calcium (p<0.0001) (table 5).

Discussion

Dental caries is a chronic disease which affects teeth and is considered globally most widely spread disease in humans. Caries occurs as a result of a complex interaction between cariogenic bacteria which produce acids and fermented carbohydrates, including many other factors of the host, such as teeth and saliva, clearly within a particular time interval. The risk of occurrence of dental caries involves various factors, including the high number of cariogenic bacteria, reduced saliva flow, insufficient exposure to fluorides and other remineralizing substances, as well as insufficient oral hygiene, inadequate diet and bad socio-economic conditions[3].

Our study included 109 subjects from both sexes at the age from 12 to 15 years with maintained general and oral health, distributed in three groups according to caries intensity, i.e. a group with very low and low caries intensity - 31 subjects (28.44%), a group with moderate caries intensity - 30 subjects (27.52%), and a group with high and very high caries intensity - 48 subjects (44.04%) (table 1). From the provided data, we can observe that the group with high and very high caries intensity has the highest number of subjects.

From our data, we can observe that the mean salivary phosphate levels were 6.11±3.1mmol/L, with a minimum of 1.08mmol/L and a maximum of 14.05 mmol/L. The calcium salivary levels in our study were 1.22±0.4mmol/L, with a minimum of 0.64mmol/L and a maximum of 3.46 mmol/L (table 2).

Table 3 shows the average values of salivary phosphates, according to the three studied groups. We can notice that the highest level of phosphates was in the group with very low and low caries intensity. The statistical analysis results showed that caries intensity significantly affects phosphate concentration in the saliva ($p=0.0009$). The post-hoc analysis for inter-group comparison confirmed a statistically significant difference in salivary phosphate levels between the group with very low and low caries intensity and the group with high and very high caries intensity ($p=0.0005$). Subjects with very low and low caries intensity showed significantly higher levels of phosphates compared to the subject group with high and very high caries intensity (table 3). Our results are consistent with the results of Stanton et al.[27] The statistical analysis of the results obtained from the concentrations of phosphates in the saliva in subjects from 12 to 15 years of age does not correspond to the study by a group of authors who reported increased level of phosphates in the saliva of caries active children.19,20

From Table 4 we can conclude that the salivary calcium levels are highest in the group with very low and low caries intensity. A statistically significant difference was confirmed in the salivary calcium levels as per caries intensity ($p<0.0001$). Subjects from the group with high and very high caries intensity showed a significantly lower level of calcium compared to the subjects from the group with very low and low caries intensity ($p=0.000001$), and a significantly lower level of calcium compared to the subjects with moderate caries intensity ($p=0.0014$) (table 4). The statistical analysis of our results regarding the calcium ion concentrations in the saliva of our subjects corresponds to the study by Aruna et al1[8], which reported increased levels of calcium in the saliva of caries resistant children and another study by a group of authors who discovered that persons with higher concentrations of calcium in the saliva have more intact teeth and are less prone to dental caries[21]. However, our results do not correspond to the study by Turtola et al.[16], and Elizarova and Petrovich[28], who reported increased concentration of calcium ions in the saliva in children with increased caries activity.

The study conducted by Gayathri R[15] indicates that the more severe the form of caries in children, the higher the concentration of calcium and phosphate ions in the saliva, which does not correspond to our results.

Considering the correlation between salivary calcium and phosphate ions, according to the value of Spearman's coefficient of correlation, there is a correlation between all the groups and salivary levels of calcium and phosphates in our study, which indicates that a change of caries intensity leads to a change in the concentrations of phosphates and calcium in the saliva, and vice versa ($R=0.353$, $R=0.51$ respectively) (table 5).

Jolly et al.[29] evaluated salivary calcium and phosphorus and found an increase in salivary calcium levels in caries-free children and no difference in salivary phosphorus between ECC (early childhood caries) and caries-free children. Similarly, Gandhy and Damle[30] reported an increase in inorganic phosphate level in children with rampant caries. The increase in salivary calcium levels in caries-active children could be due to the release of calcium from demineralized tooth, thereby increasing salivary calcium levels[31]. On the other hand, few studies insisted that there was no difference in salivary calcium and phosphate level in caries-free and caries-active children[32-35]. One of the possible explanations for no difference in calcium in both the groups could be due to the fact that saliva is a blood filtrate and the unaltered level of calcium in children with ECC might be due to the regulatory role of the parathyroid hormone (PTH), maintaining its level homogeneously in both ECC and caries-free children[36,37].

We should emphasize that dental caries and complications resulting thereof may cause serious problems not only for oral health, but also for the whole organism and

general health, and the quality of life of children and their families. Dental caries as a disease causes pain, psychological issues, problems with speech and consumption of food, and is a common reason for children's absences from school.

The use and analysis of saliva is not only important for dental diseases, it also has an increased importance in the field of medicine. Modern medicine uses salivary samples as an alternative for bodily fluids since it is easy to collect and has the same clinical-biochemical, pharmacological and toxicological parameters as blood. Recent studies have investigated the possibility to use saliva as a potential diagnostic fluid, to determine many components as they are determined in the blood, such as hormones, medicines, drug, and other toxic matters[38].

On that account, by using saliva as a diagnostic medium we may predict predisposition to dental caries and recommend its application in formulating preventive programs in everyday dental practice.

Conclusions

The analysis of the data obtained as a result of the established objectives of our study led us to the following conclusions:

The concentration of Calcium ions had significantly lower values in the subject group with high and very high caries intensity, compared to the subject group with moderate caries intensity and the subject group with very low and low caries intensity. There was also significant correlation of the DMFT index with the concentration of calcium ions in the saliva, which refers to the fact that DMFT index values increased with the reduction of calcium concentration, and vice versa, subjects with higher caries intensity showed lower calcium values.

The significant difference was confirmed in the phosphate concentration as per caries intensity. Subjects with high and very high caries intensity had significantly lower level of phosphates compared to the subjects with low and very low caries intensity. There was also significant correlation of the DMFT index with the phosphates in the saliva, which refers to the fact that DMFT index values increased with the reduction of phosphate concentration, and vice versa, subjects with higher caries intensity showed lower phosphate values, and vice versa.

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