

## SALIVARY UREA AND pH OF NON-STIMULATED SALIVA IN CORRELATION WITH DENTAL CARIES INTENSITY

Pavlevska Meri<sup>1</sup>, Gjorgievska Elizabeta<sup>1</sup>, Jankulovska Mira<sup>1</sup>, Saveski Mirko<sup>1</sup>,  
Poposki Bojan<sup>2</sup>

<sup>1</sup>Department of Pediatric and Preventive Dentistry, Faculty of Dentistry,  
Ss. Cyril and Methodius University, Skopje, Republic of North Macedonia

<sup>2</sup>Department of Periodontology and Oral Pathology, Faculty of Dentistry,  
Ss. Cyril and Methodius University, Skopje, Republic of North Macedonia  
*e-mail: mpavlevska@stomfak.ukim.edu.mk*

### Abstract

**Aim:** The aim of our study was to determine and compare hydrogen ions (pH) and urea in non-stimulated 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, which were 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 group with high and very high caries intensity involving 48(44.04%) subjects. We took saliva samples from the subjects and analyzed the pH and urea concentrations, using the spectrophotometric method.

**Results:** The average concentration of salivary urea was highest in the subject group with very low and low caries intensity ( $9.13 \pm 3.8$  mmol/L), followed by the group with moderate caries intensity ( $7.57 \pm 2.9$  mmol/L) and the group with high and very high caries intensity ( $7.22 \pm 3.3$  mmol/L). The post-hoc analysis for intergroup comparisons showed that this total significance was due to a significantly higher concentration of urea in the group with low and very low caries intensity as opposed to the group with high and very high caries intensity.

The caries intensity did not significantly affect the pH value of non-stimulated saliva ( $p=0.18$ ).

**Conclusion:** The concentration of urea had significantly lower values in the subject group with high and very high caries intensity, compared to the subject group with very low and low caries intensity. During our study we recorded statistically significant differences among the study groups with different caries intensity.

**Keywords:** caries intensity, saliva, salivary urea, pH of saliva

### Introduction

Scientific studies in the field of medicine continuously refer to the importance of oral health and that health in general starts with the mouth. Nowadays, good oral health is not only directed towards dental health, but, as broadly presented in the literature, it is the initial point for the general health and wellbeing of the whole body. Therefore, maintaining and improving oral health may have large systemic implications for the organism, for the prevention of pathologies, improving the quality of life of the individual, as well as improving the health condition in the society<sup>[1]</sup>.

The oral cavity with its defensive mechanisms prevents bacterial invasion of the organism and imbalance of the homeostasis of the oral ecosystem. For this, the host has three main barriers: oral mucosa, which covers the oral cavity and serves as a physical barrier; nonspecific (congenital) immunity and specific (acquired) immunity.

Saliva plays an important role in the maintenance of oral homeostasis, it is a product of the three pairs of large salivary glands: parotid glands, submandibular and sublingual glands and the small salivary glands which are widely distributed in the oral mucosa. Different types of acinar cells in these glands are producing different types of saliva.

Saliva as a fluid is the result of exocrine secretion, composed of approximately 99% water, non-organic part composed of electrolytes (sodium, potassium, calcium, chlorine, magnesium, bicarbonates, and phosphates) and organic part<sup>[2]</sup>.

The organic part of the saliva consists of products that are secreted in the body (urea, uric acid and creatinine), putrefaction products (putrescine cadaverine), lipids (cholesterol and fatty acids) and over 400 types of proteins. All these components are responsible for different functions of the saliva<sup>[2]</sup>.

Saliva as a specific type of biological fluid and a unique medium of the oral cavity plays an important role in the development of dental caries<sup>[3]</sup>.

Its protective role against dental caries may be summarized into four aspects, as follows:

1. Dilution and elimination of sugars and other substances from the dental surfaces,
2. Buffer capacity,
3. Balancing the processes of demineralization and remineralization,
4. Antimicrobial effect<sup>[4]</sup>.

Disruption of the balance among these factors leads to occurrence of dental caries.

Dental caries is considered the most common disease in people after the common flu. It is a multi-factor, chronic disease of the hard dental tissues, characterized by 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<sup>[5]</sup>.

Dental caries is the result of disbiosis of the dental biofilm which attaches to the surface of dental enamel. Overexposure to dietary carbohydrates leads to accumulation of microorganisms which produce acids in the mouth.

Dental caries is without a doubt a public health problem, and it is among the most widely spread global diseases connected with dental biofilm<sup>[6]</sup>.

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 promotion of public health<sup>[7]</sup>.

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 the absence of only one of these factors cannot cause caries. The effect of food on the incidence of caries is presented with the effect on the pH value of dental biofilm. The food rich in fermenting carbohydrates (mainly sugars) leads to decrease of the biofilm pH value, while food rich in protein and fat neutralizes biofilm pH value. Food rich in protein increases the concentration of salivary urea, thus increasing the pH value of biofilm, which reduces the risk of occurrence of caries. The main dietary factor that affects prevalence and progression of dental caries is saccharose<sup>[8]</sup>.

For the first time, saliva started to be used as a biological medium in the diagnostics of different diseases in the second half of the 20<sup>th</sup> century. Its main advantage is easy and noninvasive sampling, which enables early detection of particular diseases and monitoring

during the disease in correlation with treatment. Also, saliva may help detect presence of various addiction causing drugs.

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 it in sufficient amounts for further analysis. Due to the non-invasiveness of the saliva collection procedure, patients are less anxious and uncomfortable, which makes it easy to repeat sampling and monitor the disease over time. Saliva is also easier to handle during the diagnostic procedures, because it does not clot, which reduces the need of additional manipulation. It greatly affects initiation, maturing and metabolism of the dental plaques<sup>[9]</sup>.

The amount of secreted saliva, the characteristics of the saliva and its buffer capacity are factors involved in the maintenance of normal balance of the oral environment. Each change in these characteristics may influence the process of demineralization and consequently cause development of dental caries. The salivary components have important implications in reducing the risk factors involved in the incidence of dental caries<sup>[10]</sup>.

The buffering capacity of the saliva plays an important role as a mediator between the surface of the tooth and biofilm, showing its protective role against the development of dental caries. This specific characteristic of saliva is represented by the concentration of bicarbonate ion. The role of the buffer effect is in the reduction of forming acids in the dental biofilm<sup>[11]</sup>.

The composition of the saliva greatly contributes to the maintenance and increasing the pH value of the biofilm. One of the main components, which efficiently determine pH value, is sialine (which contains arginine and lysine) and urea. Further hydrolysis of these molecules releases ammonia which participates in the increase of pH value. For the maintenance of oral health and integrity of dental surfaces, the pH value should be kept at around 6.7. The concentration and activity of ions are responsible for the processes of demineralization and remineralization through the solubility of hydroxyapatite. The critical values of pH are values of 5.5 or under 5.5<sup>[12]</sup>.

The importance of the pH value of saliva in the etiology of dental caries is based on the fact that in case of a low pH value, acidophilic microorganisms survive and multiply, which significantly increase the risk of occurrence of caries<sup>[13]</sup>.

According to literature, the pH value at rest may predict the status of caries of a patient and the buffer capacity of the saliva. Patients with pH value of saliva of approximately 7.0 at rest are considered to have lower caries activity compared to patients with pH values of 5.5 who presented higher caries incidence. In individuals with pH value between 5.5 and 7.0 the incidence of caries is lower. Low pH value at rest suggests exposure to carbohydrates with prolonged maintenance of the pH value at low level. These facts suggest that in case of exposure to low pH value, teeth are in an acid environment for a prolonged period of time, without the possibility for neutralization<sup>[14]</sup>.

Varying pH of saliva leads to changes in the dental and periodontal health. Hence, the pH of saliva at 7.0 means a healthy dental and periodontal health. At this pH value, the incidence of dental caries is small, with small amount or absence of calculus; pH values of saliva under 7.0 indicate acidemia (abnormal blood acidity). If this condition persists for a longer time, conditions will be created in the mouth for development of caries, halitosis and periodontitis. In addition, chronic acidemia may be a predisposing factor for development of various diseases of the whole body. The pH value of saliva above 7.0 usually indicates alkalinity in the oral cavity and may cause the same anaerobe conditions as with acidemia, although that is a rare condition<sup>[9]</sup>.

González-Aragón Pineda AE. *et al.* in their study conducted with adolescents at the age from 12 to 14 years, analyzed the amount of secreted non-stimulated saliva, and also the pH of saliva and its buffer capacity. Subjects were divided into two groups according to DMFT

index (DMFT $\geq$ 5 and DMFT $<$ 5). The results obtained showed that the average amounts of secreted saliva, salivary pH value and the buffer capacity were significantly lower ( $p<0.05$ ) in subjects with DMFT index  $\geq$ 5 compared to subjects with DMFT index  $<$ 5. The amount of secreted saliva and salivary buffer capacity were higher in boys compared to girls<sup>[15]</sup>.

Pyati SA. *et al.* conducted a similar study involving children from 6 to 12 years of age, and they compared the values of the amount of secreted saliva, the salivary pH value, buffer capacity, total protein, malondialdehyde (MDA) and total antioxidative capacity, in the caries active group (DMFT  $\geq$ 5) and caries resistant subject group (DMFT  $<$ 5). They reported that the average amount of secreted saliva, salivary pH value and buffer capacity were significantly decreased ( $p<0.05$ ) in the caries active subject group compared to caries resistant subject group. On the other hand, MDA and total antioxidative capacity of the saliva were significantly increased in the caries active group compared to the caries resistant group<sup>[16]</sup>.

The pH value is directly connected with the concentration of calcium and phosphates ions. Critical variations of the pH value depend on many factors and it is an individual value, since salivary concentrations of calcium and phosphates vary from one person to another<sup>[17]</sup>.

Urea is part of the salivary buffer system and is involved in acid neutralization in the oral cavity. It is present in the blood and saliva as an organic substance which synthesizes from the amino acids and carbon dioxide. The importance of urea is explained by its dual role: it inhibits multiplication and metabolism of bacteria and indirectly influences acid neutralization in the oral cavity. With such action, it is involved in the salivary buffer system by maintaining acid- base balance of the saliva<sup>[18]</sup>.

The concentration of salivary urea is around 4 mmol/l and it is a bit lower than its values in the plasma. Urea may permeate from the saliva into the dental plaque, where bacterial ureases turn it into carbon dioxide and ammonia, which in turn increases the pH value. In absence of urea, the minimum pH value according to the Stephan's curve would drop for around 0.5 pH units. Uremia patients develop less caries, more calculi, and the pH value of their plaque may be as high as pH 9<sup>[19]</sup>.

Urea and arginine metabolize fast, due to the action of oral bacteria, and lead to increase of the pH value in the oral environment. Thus, patients with chronic renal failure, who have 50 times higher levels of salivary urea compared to healthy people, rarely develop caries even though their diet mainly consists of carbohydrates. Urea is one of the main sources of alkalis in the mouth, and concentrations in the secretions from all salivary glands range from 3 to 10 mM in healthy individuals. *Streptococcus salivarius* is almost the most ureolytic oral microorganism, although *Actinomyces naeslundii* and oral haemophili also present ureolytic activity<sup>[20]</sup>.

Data from literature have recently shown that urease activity in dental plaque in people without caries is around three times higher compared to caries active people<sup>[21]</sup>.

A mathematical model was invented to present the effect of urea on dental plaque in non-stimulated saliva. Accordingly, it was determined that in non-stimulated saliva, urea significantly affects dental plaque pH value by neutralizing and increasing the pH value of the plaque when the person is not eating<sup>[22]</sup>.

Zabokova BE. *et al.* in their study determined the salivary urea concentration in subjects with different DMFT index values, measured at different intervals after speaking and eating (after 5, 30 and 60 min.). According to the obtained results, the salivary urea concentration was the same in all intervals after eating and continuously and significantly decreased from its base values, whereas the lowest values were obtained 60 minutes after the meal. Furthermore, subjects with lower DMFT index showed higher salivary urea values, both for the base values and stimulated saliva values, in all intervals<sup>[18]</sup>.

Raj G. *et al.* in their study conducted with students, determined concentrations of urea and uric acid in caries active and caries resistant individuals. Their study results showed

significantly higher urea values in persons without caries as compared to caries active individuals. Proof of this is the generation of ammonia, which plays an important role in the balance between the demineralization and remineralization processes, a key role in the homeostasis of dental plaque pH and prevention of occurrence of cariogenic microorganisms that would cause development of carious process.

### **Aim**

The aim of our study was to determine and compare hydrogen ions (pH) and urea in non-stimulated saliva in patients with varying caries intensity.

### **Material and methods**

This study involved 109 subjects from both sexes, aged from 12 to 15 years, with maintained general and oral health, from the primary schools "Petar Pop Arsov" and Dimitar Pop-Georgiev Berovski", the first-year students of the secondary Medical School "Dr. Panche Karagjov", and patients from the Clinic for Pediatric and Preventive Dentistry and Clinic for Orthodontics in Skopje. Permission was obtained from the school authorities, as well as written consent from the parents for including subjects in this study.

The following were the criteria for inclusion of the subjects in the study: children with permanent dentition, at the age from 12 to 15 (we avoided mixed dentition because caries in primary teeth may compromise the results), without localized or systemic disease tackling saliva secretion, permanent residents of the city of Skopje who regularly consume local water.

Exclusion criteria were as following: children who could not cooperate during the examination and collection of material.

The subjects were assigned into three groups according to the caries intensity, i.e., according to DMFT index values (WHO, Geneva, 2000). According to the criteria of the World Health Organization (WHO), interpretation of the DMFT index values for this age group was conducted in the following manner:

- Subjects with DMFT index values between 0.0-2.4 were assigned to a category with very low and low caries intensity;
- Subjects with DMFT index values between 2.5-3.8 were assigned in the category of 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.

During collection of unstimulated saliva, subjects were advised to refrain from eating and drinking at least 60 minutes before saliva collection, as well as to avoid swallowing and oral movements during collection. To minimize the effects of diurnal variations in saliva production, saliva samples were collected in the morning, from 9:00 a.m. to 12:00 p.m. Before saliva collection, each subject rinsed his/her mouth with distilled (deionized) water.

The collection of unstimulated saliva was carried out by the spitting method, according to the recommendations of Navazesh.

The subject was seated in an upright position, with the head tilted forward, so that the saliva produced collected on the floor of the mouth, during which the collected saliva was spit into a plastic graduated tube, in a period of 10 minutes. The collected saliva was as soon as possible, with a cold chain, transferred to the Biochemical Laboratory of the Department of Oral and Periodontal Diseases of the Faculty of Dentistry - Skopje at Ss. Cyril and Methodius University in Skopje.

Before the processing phase of the collected saliva, the amount of secreted unstimulated saliva and the concentration of hydrogen ions (pH value) were determined.

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

**Materials for the laboratory tests:**

We used the following materials for the clinical trial: sterile dental probe and mirror, plastic single use glasses, plastic single use tubes (for collecting saliva), Eppendorf single use tubes (for saliva analyses), deionized water, spectrophotometer (BIOBASE/BK-UV1000 Spectrophotometer), centrifuge (BIOBASE-High Speed Refrigerated Centrifuge), vortex device (DRAGONLAB MX-S), protective single use gloves, pipettes and tips for pipettes.

For the spectrometric measurements of the salivary urea, we used BioSystems reagents UREA/BUN-UV.

All materials and reagents used were with the relevant degree of purity necessary for analysis.

**Clinical trials**

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

Determination of the DMFT index using Klein-Palmer system was performed by summarizing the total number of decayed, extracted and restored-filled permanent teeth.

$$\text{DMFT} = \text{decayed teeth} + \text{missing due to caries} + \text{filled teeth}$$

**Determination of the concentration of hydrogen ions (pH value)**

Determination of the hydrogen ions concentration (pH value) was the last phase before processing the collected saliva. Before conducting the measurements, first we calibrated the pH meter (pH-meter PH-03(II) Pen-type pH Meter), using standard buffer solutions: Certipur pH 4.00 (Buffer Solution: Citric acid/Sodium hydroxide/Hydrogen chloride) and Certipur pH 7.00 (Buffer Solution: Potassium dihydrogen Phosphate/di-Sodium Hydrogen Phosphate).

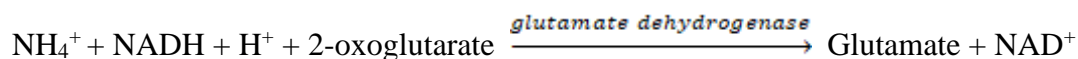
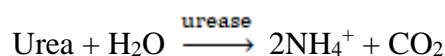
**Processing the collected saliva**

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 a centrifuge (BIOBASE-High Speed Refrigerated Centrifuge). After centrifuging, we collected 500-1000  $\mu\text{l}$  of the supernatant of the centrifuged saliva, using micro-pipette, and distributed the sample into small plastic single use tubes (Eppendorf tubes). The processed saliva samples were frozen at  $-20^{\circ}\text{C}$ , and the analysis of samples was conducted at latest 15 days from the date of freezing.

The next phase of our study was to determine concentrations of salivary urea.

The analysis was made upon fast defrosting of the samples.

- Salivary urea was determined according to the UREA/BUN-UV method, with double reaction, while urea under the influence of urease enzyme hydrolyzes in ammonia and carbon dioxide. Urea released ammonia then reacts with NADH and 2-oxoglutarate in presence of the glutamate dehydrogenase enzyme (GLDH) resulting in glutamate and  $\text{NAD}^+$ . We made the measurements using a spectrophotometer, at wavelength of 340 nm.



### Results

The study involved 109 subjects, distributed in 3 groups according to the 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 group with high and very high caries intensity involving 48 (44.04%) 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 (urea and concentration of hydrogen ions).

**Table 2.** Statistical parameters of salivary markers

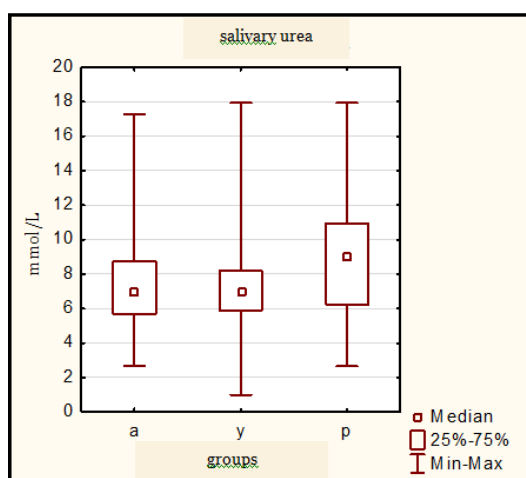
Parameter	Descriptive statistics		
	mean ± SD	min - max	median (IQR)
Urea (mmol/L)	7.92±3.4	1-17.92	6.98(5.89-9.29)
pH value	7.29±0.3	6.45-7.89	

The average concentration of salivary urea was highest in the subject group with very low and low caries intensity (9.13±3.8 mmol/L), followed by the group with moderate caries intensity (7.57±2.9 mmol/L) and the group with high and very high caries intensity (7.22±3.3 mmol/L).

**Table 3.** Values of salivary urea – groups according to caries intensity

Groups	Descriptive statistics- Urea (mmol/L)		p-value
	mean ± SD	median (IQR)	
p	9.13±3.8	8.94(6.22-10.95)	H=6.1 *p=0.046 post-hoc p vs a *p=0.048

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<0.05



**Fig. 1.** Median urea value - groups according to caries intensity

The median value of the concentration was also highest in the group with low and very low caries intensity (8.94 mmol/L), followed by the groups with moderate caries intensity and high and very high caries intensity (6.94 mmol/L and 6.915 mmol/L), respectively (Table 3, Figure 1).

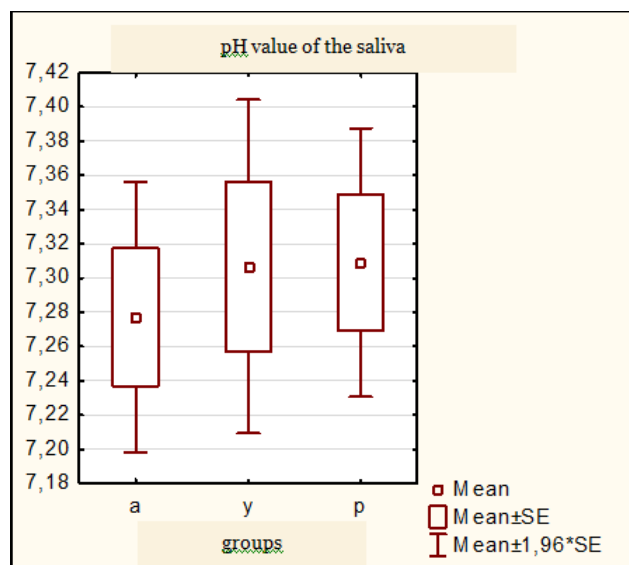
For  $p=0.046$  we established a total statistically significant difference in salivary urea concentrations, as per caries intensity. The post-hoc analysis for intergroup comparisons showed that this total significance was due to significantly higher concentration of urea in the group with low and very low caries intensity as opposed to the group with high and very high caries intensity ( $p=0.048$ ) (Table 3).

Caries intensity did not significantly affect the salivary pH value ( $p=0.18$ ). The average concentration of hydrogen ions was similar, i.e., insignificantly different in the three groups ( $7.28\pm0.3, 7.31\pm0.3$ , and  $7.31\pm0.2$ , respectively, in the groups with very high and low caries intensity, moderate and high and very high caries intensity) (Table 4, Figure 2).

**Table 4.** pH values – groups according to caries intensity

Groups	Descriptive statistics- pH		p-value
	mean $\pm$ SD	min - max	
p	7.28 $\pm$ 0.3	6.45-7.76	F=0.18 p=0.83
y	7.31 $\pm$ 0.3	6.59-7.89	
a	7.31 $\pm$ 0.2	6.81-7.8	

p-group with very low and low caries intensity, y-group with moderate caries intensity, a-group with high and very high caries intensity, F-Analysis of variance



**Fig. 2.** Mean pH value of saliva-groups according to caries intensity

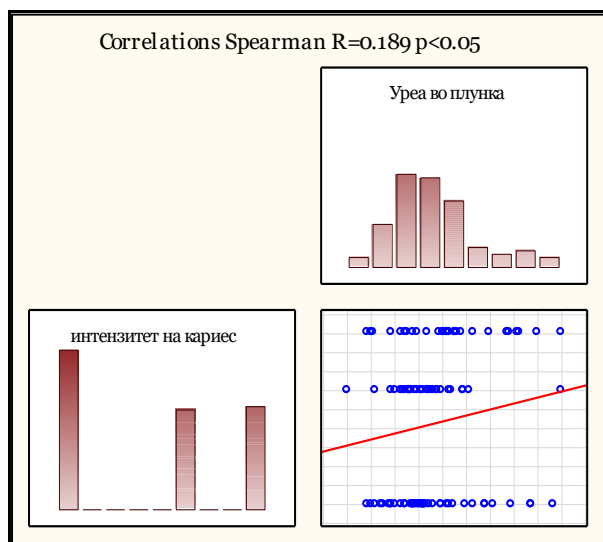
The analysis of caries intensity with salivary biomarkers showed that the intensity of caries significantly correlated with the concentration of salivary urea ( $p=0.049$ ) (Table 5).

**Table 5.** Correlation-caries intensity with salivary biomarkers

Caries intensity	Correlation		
	Spearman R	T	p-value
Urea (mmol/L)	0.189	1.987	*0.049
pH value	0.055	0.573	0.57

\* $p<0.05$ , \*\*\* $p<0.0001$





**Fig. 3.** Correlation-caries intensity with salivary urea

According to the Spearman's coefficient of correlation, all these correlations were positive, i.e. direct, which indicated that increase of the caries intensity led to increase in the concentrations of salivary urea, and *vice versa* ( $R=0.189$ , respectively) (Table 5, Figure 3).

We used the statistical program SPSS 23.0, Kolmogorov-Smirnov test and Shapiro Wilk's test for the statistical analysis of data obtained in the study, to test the regularity of data distribution.

The category (attribute) related variables are shown in absolute and relative figures. Numerical (quantitative) variables are presented as average, standard deviation, minimum and maximum values, median values and interquartile ranking.

For the comparison of the three groups of caries intensity to the salivary biomarkers, we used non-parameter tests for independent samples (Kruskal-Wallis test, Mann-Whitney test, Analysis of variance test).

The correlation between the caries intensity and salivary biomarkers was analyzed using non-parameter correlation (Spearman's rank correlation coefficient).

Statistical significance was defined as a p value  $<0.05$ .

### Discussion

Dental caries is a chronic disease which affects teeth and it is considered globally the 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 great number of cariogenic bacteria, reduced saliva flow, insufficient exposure to fluorides and other remineralizing substances, insufficient oral hygiene, inadequate diet and bad socio-economic conditions.

According to the US Department of Health and Human Services, 2014, dental caries is the most frequent chronic disease in children, five times more frequent than asthma and seven times more frequent than seasonal allergies<sup>[24,25]</sup>.

Our study involved 109 subjects from both sexes at the age from twelve to fifteen years, with maintained general and oral health, distributed in three groups according to caries intensity, i.e., group with very low and low caries intensity, group with moderate caries intensity and group with high and very high caries intensity.

Early detection of diseases is of vital importance for mediating the severity of the disease and prevention of complications, and also, it is of essence for increasing therapeutic success rate. During the last decade, saliva has been largely researched as a potential diagnostic tool due to easy and noninvasive sampling thereof, but also, due to the availability of numerous salivary biomarkers<sup>[26]</sup>.

There is literature on the influence of saliva in the protection of teeth through its components and functions in: buffering capacity, the self-cleaning effect, antibacterial effect and maintenance of calcium and phosphates saturation. Knowing the functional characteristics of the saliva, as well as its integral components, it is possible to better assess the sensitivity to dental caries<sup>[267]</sup>.

One of the aims of our study was to determine the pH value of non-stimulated saliva in the subject group with different caries intensity.

The salivary pH value was similar in the three groups, i.e., non-significant difference in salivary pH among subject groups with low and very low, moderate and high and very high caries intensity ( $7.28 \pm 0.3$ ; vs.  $7.31 \pm 0.3$ ; vs.  $7.31 \pm 0.2$ ;  $p=0.83$ ).

These salivary pH values do not correspond to the results of the studies by González-Aragón Pineda AE. *et al.*<sup>[15]</sup> and Pyati SA. *et al.*,<sup>[16]</sup> which obtained significantly lower pH values of the saliva in the subject group with low and very low caries intensity as opposed to the group with very high caries intensity.

The reason for such results for the salivary pH may be that sampling was done in the mornings, and the subjects had their teeth brushed and had not eaten yet.

The latest caries-related studies focus on the fact that alkaline matter generation from the salivary substrates, such as urea and uric acid, may act as biomarkers of dental caries by maintaining pH values and inhibition and homeostasis of the biofilm<sup>[23]</sup>.

Urea is important for the regulation of acid-base balance and maintenance of salivary pH homeostasis, and influences the dental caries incidence<sup>[28]</sup>.

Production of ammonia as a result of oral bacteria significantly affects the oral microflora and it is connected to the oral health and diseases. Urea is one of the nitrogen salivary substrates, with significant effect on the formation of base environment in the oral cavity<sup>[20,29]</sup>.

Urea is hydrolyzed as a result of the urease enzyme, which is part of some oral bacteria. Ammonia is generated during ureolysis and may lead to increase of the pH value of the plaque, despite carbohydrate-rich diet.

Literature supports the fact that the risk of caries incidence is directly connected with the loss of the potential to generate alkalis. Thus, alkaline potential of the biofilm may be used as a strategy for dental caries control. Accordingly, caries resistant individuals have three times higher urease values in plaque samples compared to caries active individuals<sup>[30,31]</sup>.

In our study, one of the objectives was to determine the concentration of salivary urea in subjects with different caries intensity. According to the obtained results, the subject group with high and very high caries intensity showed lower concentrations of urea compared to the subject group with very low and low caries intensity. The average concentration of salivary urea was  $7.22 \pm 3.33$  mmol/L in subjects with high and very high caries intensity, and  $9.13 \pm 3.8$  mmol/L in the group with very low and low caries intensity. There was a significant difference of 6.1 mmol/L, for  $p < 0.046$ .

These results correspond with the results from the study by Zabokova BE. *et al.*<sup>[18]</sup>, which reported higher urea values in the subjects with lower DMFT index values compared to the subjects with higher DMFT index values.

Similar results were also presented in the study by Raj G. *et al.*<sup>[23]</sup>, which included students. They obtained significantly higher urea values of in students without caries compared to caries active individuals.

Discovering the risk factors as well as the low amounts of protective factors in the saliva of subjects exposed to dental caries risk facilitates disease management and provides for adequate recommendations to individuals. Introducing saliva as a diagnostic tool in the daily practice gives many benefits both for dentists and patients. Also, it is important from the aspect of improved diagnostics, early detection, improving doctor-patient communication and motivation, and increasing dental awareness of patients. In addition, it would provide for categorization of patients in groups with low, moderate and high caries activity, and would enable application of adequate preventive measures which would reduce the incidence of caries.

We should emphasize that dental caries and complications resulting thereof may cause serious problems not only for the 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, raises psychological issues, problems with speech and food consumption, and it is a common reason for children's absences from school.

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

### **Conclusion**

Saliva and its components play an important role in maintaining oral, especially dental health. Urea contributes to the maintenance of acid-base balance of the saliva, which in turn, affects caries incidence. The positive effect of urea was confirmed by the results obtained in this study: subjects with lower DMFT index presented with greater urea concentration compared to subjects with high and very high caries intensity. By regulating acidity in the saliva, urea has a buffer role, which decreases the possibility for occurrence of dental caries. The value of salivary urea may serve as a parameter for determining the risk of caries, which, in turn, may be used in planning and implementing adequate caries preventive measures.

The salivary pH value was similar in the study groups, i.e., non-significant difference in salivary pH among the subject groups with different caries intensity.

*Conflict of interest statement.* None declared.

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