CORRELATION BETWEEN SALIVARY UREA LEVEL AND DENTAL CARIES

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Abstract: Objectives: The aim of this study was to determine the values of salivary urea in subjects with different caries activity.

Material and method: The planned trials were conducted in 80 children of both sexes, aged 16 years, with different caries activity. Based on the condition of teeth, the DMFT-index, respondents were divided into two groups: the first group consisted of 40 examinees with a low to very low index of caries (0–3), the second group consisted of 40 examinees with a high index of caries (> 10). Material for biochemical testing of the saliva sample was taken from all subjects at different time intervals: 5, 30 and 60 minutes from the (daily) meal. The examined parameters were followed in the same examinees in a sample of saliva taken in the morning before consuming any food or implementation of oral hygiene: they represent basic information compared with the results of the examination. The concentration of urea in saliva was determined by the enzyme method of continuous measurement. This method is based on the principle of hydrolysis of urea, using the enzyme urease.

Results: Salivary concentration of urea, measured fasting in the morning (basic values) in examinees with a low caries index, ranging in limits from 5.50 to 9.10 mmol/l, and significantly lower values in examines with a high DMFT-index (from 3.40 to 5.50 mmol/l). The same was done with the concentration of salivary urea at different time intervals after taking the meal – 5, 30 and 60 minutes in the examinees with a different DMFT-index. With the increasing time interval after taking a meal, the concentration of salivary urea continuously and significantly declines compared to its baseline concentration. The largest decrease of concentration of urea in terms of its basic value in all examinees with a different DMFT-index (with low and high) took place during the 60 minutes after having the meal.
Conclusion: Saliva with its constituents plays an important role in maintaining oral, and especially dental health. Urea contributes in maintaining the acidobasic balance of saliva, and thus affects the incidence of caries. The positive effect of urea was confirmed by the values found in this study: the respondents with a lower DMFT-index present a higher concentration of urea than in the basic values, and in the values of stimulated (through the meal) saliva, followed in all intervals.

Key words: saliva, salivary urea, dental caries.

Introduction

Saliva is a biological environment, important for the physiology of the mouth. It achieves its mechanical functions of cleaning and protection through various physical and biochemical mechanisms. For keeping the electrochemical reaction in oral homeostasis, except the bicarbonate, phosphate and protein buffer, other compounds or enzymes participate, having a buffer role. This group includes urea, salivary amylases and fluorides as prophylactic buffers.

The importance of salivary urea was acknowledged early in dental literature [11, 12]. The pH-raising effect of intraoral urea application was first described by Stefan [22]. This author found that in both in vivo and in vitro urea could raise plaque pH up to pH 9 and that the addition of 40–50% urea to carbohydrates largely overcame the pH-lowering effect for up to 24 h.

The value of salivary urea ranges from 2 to 6 mmol/l. Urea has a dual effect: it inhibits the metabolism and multiplication of bacteria in the saliva on the one hand, and on the other hand it indirectly affects neutralizing the acids in the oral environment, thus participating in maintaining the salivary acidobasic balance, which it actually owes to its buffer capacity [1, 8]. Urea entering the mouth is hydrolyzed to carbon dioxide and ammonia by bacterial ureases. Ammonia production from arginine and urea metabolism has been identified as a mechanism by which oral bacteria: (1) are protected against acid neutralizing, (2) maintain a relatively neutral environmental pH that may suppress the emergence of cariogenic microflora and (3) derive bioenergetic advantages, including increasing pH and, for arginine specifically, synthesizing adenosine triphosphate [9, 10, 17, 18].

For neutralises acids, urea can be used as a constituent of chewing gums. The effect of sugar-free chewing gums containing various amounts of urea on the pH recovery in dental plaque was researched by Imfeld [13]. After rinsing the mouth with 10 or 50% (w/v) sucrose solution, the respondents chewed the gum with different content of urea (10, 20, 30 mg) for 10 minutes. Increased value of salivary or plaque pH was found in the first minutes of chewing, and the
effect of urea continued and lasted over ten minutes. The higher concentrations of urea in chewing gum resulted in a faster levelling of the pH. Thus, the highest values of pH in the examined groups were observed in cases where they were treated with chewing gum containing 30 mg urea. With the use of such chewing gum the salivary pH value does not fall below the level which is risky for the occurrence of dental caries, and there is a positive effect of chewing on the salivary flow that also affects neutralizing the acids in saliva or plaque [3].

Urea, which is present in blood and saliva, is an organic substance synthesized from amino acids and carbon dioxide. Some oral microbes hydrolyze salivary and dietary urea via the enzyme urease to produce ammonia and carbon dioxide, which results in an increase in plaque pH [5, 7]. A mathematical model of the influence of salivary urea on dental plaque was constructed to demonstrate the effect it can have on unstimulated saliva. Data from study 52 indicated that urea present in unstimulated saliva has a significant effect on plaque pH by elevating and counteracting the fall of plaque pH in the fasting state [4].

The correlation of higher salivary urea concentrations and low salivary caries activity was registered in patients with chronic renal disease. These patients, who have elevated salivary urea concentration, have a reduced incidence of dental caries [24].

The purpose of this study was to determine the values of salivary urea in examinees with different caries activity.

Material and method

The planned trials were conducted in 80 children of both sexes, aged 16 years, with different caries activity. They had good general health and were not included in a fluoride prophylactic programme. Based on the state of teeth, the DMFT-index, examinees were divided into two groups:

– the first group consisted of 40 examinees, with a low to very low index of caries (0–3),
– the second group consisted of 40 examinees, with a high index of caries (> 10).

The following tests were made:

– clinical trials,
– biochemical tests on saliva.

The clinical examination of participants was carried out: determination of the DMFT-index. Material for biochemical testing the saliva sample was taken from all examinees at different time intervals: 5, 30 and 60 minutes after the (daily) meal. The examined parameters were followed in the same exami-
nees in a sample of saliva taken in the morning before consuming any food or implementation of oral hygiene: they represent basic information compared with the results of the examination.

The concentration of urea in saliva was determined by the enzyme method of continuous measurement. The method is based on the principle of hydrolysis of urea, using the enzyme urease. Ammonia is obtained, which reacts with the acid \( \alpha \)-oxoglutarate and NADH\(_2\) (the catalytic effect of GLDH-glutamat dehidrogenaza), which occurs in glutamic acid and NAD. The fall of absorption due to oxidation of reduced NAD is proportional to the presence of ammonia liberated from urea [25]:

\[
\text{Urea} + 2\text{H}_2\text{O} \xrightarrow{\text{Urease}} 2(\text{NH}_4)_2\text{CO}_3
\]

\[
2\alpha-\text{oxoglutarate acid} + 2\text{NH}_4^+ + 2\text{NADH} + H^+ \xrightarrow{\text{GLDH}} 2\text{glutamic acid} + 2\text{NAD}^- + 2\text{H}_2\text{O}
\]

The concentration of urea was measured at a temperature of 37°C, wavelength 340 nm and is expressed in mmol/L.

The results obtained from previously performed tests were analysed using statistical parameters: mean (\( \bar{x} \)), Standard deviation (SD), Standard error (SE), Student t-test.

**Results**

The value of basic salivary urea ranges from 5.50 to 9.10 or, on average, from 7.32 in the first group and from 3.40 to 5.50 or, on average, 4.65 in the second group of examinees (Table 1 and Graph 1).

Table 1

<table>
<thead>
<tr>
<th>group</th>
<th>( \bar{x} )</th>
<th>SD</th>
<th>SE</th>
<th>min</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7.32</td>
<td>1.0339</td>
<td>0.1888</td>
<td>5.50</td>
<td>9.10</td>
</tr>
<tr>
<td>II</td>
<td>4.65</td>
<td>0.5800</td>
<td>0.1059</td>
<td>3.40</td>
<td>5.50</td>
</tr>
</tbody>
</table>

\( t = 12.305; \text{df} = 58; p < 0.01 \)
Table 2 and Graph 2 are review values of salivary urea in both groups of examinees 5 minutes after the daily meal. The average value of salivary urea in the first group of respondents was 5.71, with minimum values of 3.90 and maximum values of 7.50, while in the second group of examinees the average value of urea in saliva was 4.67, with minimum values of 4.20 and maximum values of 5.20. A highly significant difference (p < 0.01) was found between both groups.

Table 2

<table>
<thead>
<tr>
<th>group</th>
<th>$\bar{X}$</th>
<th>SD</th>
<th>SE</th>
<th>min</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5.71</td>
<td>1.0298</td>
<td>0.1880</td>
<td>3.90</td>
<td>7.50</td>
</tr>
<tr>
<td>II</td>
<td>4.67</td>
<td>0.3415</td>
<td>6.236E-02</td>
<td>4.20</td>
<td>5.20</td>
</tr>
</tbody>
</table>

t = 5.267; df = 58; p < 0.01
Table 3 and Graph 3 show the values of the concentration of urea in saliva taken 30 minutes after the daily meal. The value of salivary urea ranges from 3.20 to 6.50 or, on average, from 2.15 in examinees with a low caries index and from 3.60 to 4.80, or an average of 4.02, in the examinees with a high index of caries. The noticeable difference is highly significant (p < 0.01) between the two groups.

Table 3

<table>
<thead>
<tr>
<th>group</th>
<th>$\bar{x}$</th>
<th>SD</th>
<th>SE</th>
<th>min</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5.21</td>
<td>0.901</td>
<td>0.1789</td>
<td>3.20</td>
<td>6.50</td>
</tr>
<tr>
<td>II</td>
<td>4.02</td>
<td>0.3788</td>
<td>6.916E-02</td>
<td>3.60</td>
<td>4.80</td>
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</tbody>
</table>

$t = 6.203; \; df = 58; \; p < 0.01$
Table 4 and Graph 4 review the values of salivary urea in the respondents 60 minutes after the daily meal. The average value of salivary urea in the first group of examinees, who had a low index of caries, is 4.58, with minimum values of 3.20 and maximum of 6.10, while the second group of examinees, who had a high caries index, the mean value of urea in saliva was 3.54, with minimum values of 3.30 and maximum values of 3.90. The value of salivary urea in this time-interval shows a highly significant difference, $p < 0.01$.

Table 4

<table>
<thead>
<tr>
<th>group</th>
<th>$\bar{X}$</th>
<th>SD</th>
<th>SE</th>
<th>min</th>
<th>max</th>
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</thead>
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<tr>
<td>I</td>
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<td>0.9849</td>
<td>0.1798</td>
<td>3.20</td>
<td>6.10</td>
</tr>
<tr>
<td>II</td>
<td>3.54</td>
<td>0.2008</td>
<td>3.667E-02</td>
<td>3.30</td>
<td>3.90</td>
</tr>
</tbody>
</table>

$t = 5.685; \text{df} = 58; \ p < 0.01$

Прилож. Одз. біол. мед. наук. XXXIII/1 (2012), 289–302
Table 5 shows the values of salivary urea in all time periods of examination – 5, 30 and 60 minutes after the daily meal, and also shows the significance of the differences in the value of salivary urea between the two groups. The reduction in the value of salivary urea can be determined in both groups at all time intervals, compared to the basic value. Differences in average values of salivary urea in terms of its basic value in the first examined group were: 1.60 (5 minutes after daily meal), 2.11 (30 minutes after daily meal) and 2.74 (60 minutes after daily meal). Differences in average values of salivary urea in terms of its basic value in the second examined group are: 1.67E-02 (5 minutes after daily meal), 0.64 (30 minutes after daily meal) and 1.12 (60 minutes after daily meal) (Graph 5).

### Table 5

<table>
<thead>
<tr>
<th>group</th>
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<th>5 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7.32</td>
<td>5.71</td>
<td>5.21</td>
<td>4.58</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>1.60**</td>
<td>2.11**</td>
<td>2.74**</td>
</tr>
<tr>
<td>II</td>
<td>4.65</td>
<td>4.67</td>
<td>4.02</td>
<td>3.54</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>1.67E-02*</td>
<td>0.64**</td>
<td>1.12**</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01
Graph 5 – Values of salivary urea at 5, 30 and 60 minutes after consuming meal

Graph 6 gives the differences in average values of urea in saliva at certain intervals: 5, 30 and 60 minutes, compared to the basic value. The difference is greatest in the time interval of 60 minutes after the meal.

Graph 6 – Differences in average values of urea in saliva at certain intervals of 5, 30 and 60 min compared to the basic value

Discussion

Saliva is the most important biological factor in the etiology of the occurrence of caries, reducing and removing erosion substances from the mouth. Among the numerous properties of saliva (buffer capacity, the contents of calcium, phosphate, mucin, etc...), it can be considered that the most significant
in the occurrence of dental caries are the volume of secretion of unstimulated saliva and the buffer capacity. A small amount of released, unstimulated saliva has a lower pH value and a lower buffer capacity, or less ability to reduce, remove and neutralize acidic food products [2, 16, 19, 23, 26]. In the oral cavity, as an initial part of the digestive system, the substances are imported through food and affect the concentration level of hydrogen ions (pH) in saliva. Thanks to a salivary buffer system, this level is maintained within certain narrow limits (from 6.8 to 7.2) [15].

The availability of carbohydrates and nitrogen substrates in food which are constituents of saliva, the speed of salivary secretion, its buffer capacity, the presence of calcium and phosphate, together with plaque microorganisms, affect the metabolic activity of plaque on the pH value and, ultimately, its tendency to demineralise the hard dental tissue [6, 14].

The buffer capacity of saliva has a direct and important impact on the time-interval required for the establishment of normal acid of the saliva. The role of buffer maintenance of acido-basis balance, or oral homeostasis, is one of the most important natural protective functions of saliva.

Urea is a part of the buffer system in saliva, which participates in neutralizing the acids in the oral environment. The different concentrations of urea, determined in this work in groups with different DMFT-indexes, point to its role in the occurrence of caries. Differences in the concentration of urea refer to the basic values and to the values of salivary stimulation (the act of mastication and effect of food).

Salivary concentration of urea measured fasting in the morning (basic values) in examinees with a low caries index, ranged in limits from 5.50 to 9.10 mmol/l, and had significantly lower values in examinees with a high DMFT-index (from 3.40 to 5.50 mmol/l). The concentration of salivary urea at different time intervals after taking the meal – 5, 30 and 60 minutes in the subjects with different DMFT-indexes is the same in terms of the concentration. By increasing the time-interval after taking a meal, the concentration of salivary urea continuously and significantly declines relative to its baseline concentration. The largest decrease of concentration of urea in terms of its basic value in all examinees with different DMFT-indexes (low and high) takes place during the 60 minutes after taking a meal.

This result confirms the notion that salivary urea participates in neutralizing the acids in saliva, keeping the pH value to a certain level. This finding is yet another confirmation of the (direct) role of salivary urea in maintaining the acidobasic balance, which is important for oral equilibrium.

The findings on the concentration of salivary urea obtained in this study are in accordance with the findings of researchers in this field – Singer, Imfeld [13, 20]. However, the results in terms of time-interval may be compared only
with those of Singer, whose research relates to a period of 5, 10 and 20 minutes after taking a meal; the final results of the author lead to the same conclusion, that salivary urea has an important role as a buffer on the occurrence of caries, i.e. on dental health [21].

An interesting finding is that of Imfeld [13], who compared the action of urea and sodium bicarbonate after rinsing the mouth with a solution of them, finding that the effect of synthetic urea is greater and more lasting than that of sodium bicarbonate: during rinsing with a solution of sodium bicarbonate, higher pH values were observed, but values of the plaque pH began to decline shortly after spitting, which was not the case with the urea. This finding the author explains by the direct, rapid penetration of urea into the plaque, and the slow disappearance of the same; it is considered that this characteristic of urea is not due to its diffuse power, but to its antibacterial effect.

Conclusion

The saliva with its constituents plays an important role in maintaining oral and especially dental health.

The urea contributes to maintaining the acidobasic balance of saliva, and thus affects the incidence of caries. The positive effect of urea was confirmed by the values found in this study: the respondents with a lower DMFT-index presented a higher concentration of urea than in the basic, and in the values stimulated (through the meal) saliva, followed at all intervals.

Regulating salivary acidity, urea performs the role of a buffer, reducing the possibility of the occurrence of dental caries. The value of urea in saliva in adolescence can serve as a parameter for determining the risk of caries, and this, in turn, can be used in the planning and implementation of appropriate caries-preventive measures.

REFERENCES


Резиме

НИВОТО НА САЛИВАРНАТА УРЕА ВО КОРЕЛАЦИЈА СО ЗАБНИОТ КАРИЕС

Жабокова Билбилова Е., Сотировска Ивковска А., Амбаркова В.

Апстракт: Цел: Целта на овој научен труд беше да ги дентермиираме вредностите на саливарната уреа кај испитаници со различен степен на карис активитет.

Материал и метод: Во испитувањето беа вклучени 80 испитаници од двата пола, на 16-годишна възраст кои се основа на состојбата на забите, односно КЕП-индексот беа поделен во две групи. Првата група ја сочинувава 40 испитаници со многу низок до низок индекс на карис (0-3), а втората група ја сочинувава 40 испитаници со висок индекс на карис (>10). За биохемиските испитувања материјал – примерок на плунка, беше земен од сите испитаници во различни временски интервали: по 5, 30 и 60 минути од главниот (дневен) оброк. Испитуваните параметри се проследени кај остите испитаници и во примерок на плунка земен наутро, пред консумирањето на било каква храна или спроведување на орална хигиена (базични вредности во однос на кои се споредуваат резултатите од испитувањето). Концентрацијата на саливарната уреа е одредува со елизимски метод на континуирано мереење, заснован на принципот на хидролиза на уреаза со елизимот уреаза.

Резултати: Концентрацијата на саливарната уреа, измерена наутро на гладно (базични вредности) кај испитанициите со низок индекс на карис, се движи во границите од 5,50 до 9,10 mmol/l, а синхронизирано се пониски вредностите кај испитанициите со висок карис – индекс (од 3,40 до 5,50 mmol/l). Иста е и концентрацијата на саливарната уреа во различните временски
интервали по земањето на оброкот – 5, 30 и 60 минути кај испитаниците со различен КЕП-индекс. Со зголемување на временскиот интервал по земањето на оброкот, континуирано и сигнификантно опфаќа концентрацијата на саливарната уреа, во однос на нејзината базична концентрација. Најголемо намалување на концентрацијата на уреата, во однос на нејзината базична вредност кај сите испитаници со различен КЕП-индекс (со низок и висок), се случува за време од 60 минути по земањето на оброкот.

Заклучоци: Плунката со своите конституенти има важна улога во одржувавањето на оралното, и посебно – денталното здравје. Уреата има придонес во одржувавањето на ацидабазната рамнотежа на плунката, а преку тоа, и на појавата на карисот. Позитивниот ефект на уреата го потврдуваат и вредностите констатирани во ова истражување: кај испитаниците со понизок КЕП-индекс е присутна повисока концентрација на уреа, како на базичните така и на вредностите во стимулираната (преку оброк) плунка, во сите проследени временски интервали.

Ключни зборови: плунка, саливарна уреа, забен карис.

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