Associating Certain Salivary Parameters with Oral Health for a Group of Patients with Type II Diabetes Mellitus

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This study purpose was to find out if salivary parameters of patients with type II diabetes influences oral health. 171 patients which were divided into three equal groups were included in the study (n=57). Group A included patients which were clinically healthy, group B included patients with type II diabetes and controlled glycemic parameters, and group C included patients with glycemic parameters beyond recommended values. The following parameters were determined to be: unstimulated salivary flow, salivary pH level, salivary calcium concentration, caries intensity index, fasting blood sugar and glycosylated hemoglobin. Statistical analysis of the groups showed links between salivary parameters, glycemic parameters and oral health. The strongest correlation was found to be between salivary calcium concentration (r=0.543) and fasting blood sugar levels respectively glycosylated hemoglobin (r=0.518) for a level of p<0.001.

Keywords: type II diabetes, salivary parameters, glycemic parameters, oral health

In recent decades, there has been a worldwide increase in the rate of diabetes cases. Recent published data shows that Romania ranks among the European countries with a very high prevalence of diabetes mellitus of 11.6% [1].

Modifications of pancreatic secretion are a cause of chronic hyperglycemia, as well as reducing the tissue's response to insulin, having long-term effects or modifications to a number of different tissues and organs, particularly: the eyes, kidneys, nerves, heart, blood vessels or salivary glands [1, 2]. Hyperglycemia determines anatomical modifications of salivary glands which develop functional consequences manifested by reduced salivary flow or modified salivary composition [3,4].

The consequences of these salivary modifications determine the proliferation of microorganisms that orally produce an increased number of dental caries or periodontial disease compared to non-diabetic patients [4,5]. Today saliva is regarded as a fluid that bathes the oral tissues, which favors it’s function to protect from many diseases [6-9].

An adequate calcium level which can oppose the demineralization process is one of the mechanisms in which saliva intervenes [10, 11]. Other qualities of saliva are: it minimizes the accumulation of dental plaque and opposes caries formation with its clearance and buffering capabilities [12-14].

Oral modifications for people with type II diabetes mellitus may present important functions changes that include: xerostomia, increased infection susceptibility, salivary dysfunctions or modifications in pH level [15-17]. The link between patients with type II diabetes biological parameters and oral health can be assessed by the intensity index for dental caries DMF-T (decayed, meesing, felled, tooth) but also by the gingival health status [17-20].

Although the salivary composition is influenced by an increased number of biological variables, this fluid can be compared to blood plasma which is going through a constant change. Saliva also provides an excellent alternative to serum for the following reasons: it’s easy to collect, cost of harvesting is reduced, transportation and storage in noninvasive and is also suitable for screening [20, 21].

The proposed objective of this study is to verify whether, for a group of patients with type II diabetes that are hospitalized in the diabetic section of the Clinical Emergency Hospital in Sibiu, exists significant correlations between glycemic factors and salivary factors when determining oral health. We also want to discover if salivary factors (unstimulated salivary flow, pH level and salivary calcium concentration) can be considered protective factors for oral health.

Experimental part

This study was conducted in 2015, after obtaining written consent from the Ethics Committee and written consent of patients acknowledging they were informed. 171 patients, between the ages of 40 to 79, male and female, were included in the study. Patients were randomly selected and divided into 3 groups according to glycosylated hemoglobin values (HbA1c):
- Group A: n = 57 patients which are clinically healthy,
- Group B: n = 57 diabetic patients with glycemic control having glyced hemoglobin < 8 % (HbA1c<8),
- Group C: n = 57 diabetic patients with constant glucose levels beyond recommended glycosylated hemoglobin ≥ 8 % (HbA1c ≥ 8).

Saliva was collected 2 h after breakfast between 9-11 am, and participants were asked to rinse with distilled water before giving their sample. Saliva was collected in sterile disposable containers, for a period of 5 min. In this way we determined the unstimulated salivary flow. Samples were refrigerated at a temperature of -20°C to remain preserved until chemical analyses were performed.

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The following criteria for patients with type II diabetes were included in the study: male and female patients between ages 40 and 69 and to have this disease for a minimum of 3 years, and the DMF-T level to be ≥ 6.

All patients with type II diabetes come from monitored cases by the Ambulatory Clinical Emergency Hospital of Sibiu. Patients with salivary complications caused by diabetes were excluded, xerostomia or acute inflammatory issues caused by dental problems. The dental examination was done by a single doctor using natural lighting, and all data was recorded on individual forms.

5 mL were collected from saliva samples for a 30 centrifugé at 3000 rpm to obtain the supernatant necessary for the analysis. To determine the pH of the saliva an electronic pH meter with a single electrode was used: Seven Compact PH Mettler Toledo (AutoChim Inc. USA). Salivary calcium concentration was determined using a spectroscopy-photometer T80 UV/Vis Spectrometer (PG Instruments Ltd.) respecting the instructions of the dosing kit Ca++ (BioSystems Methyl/thymol Blue).

The results were rated as averages of the values obtained ± the standard error measurement (± SEM). The statistical significance between the values of the test group and the values of the groups with type II diabetes mellitus was assessed by the method of parametric analysis of variations with a single inter-group factor (One-Way Anova), which compares the average values of quantitative variables between 2 or more groups. A critical threshold for statistical significance was considered p < 0.001. In order to measure statistically the degree of correlation between variables the coefficient r correlations was used, determined by the Person test. These analyses were conducted using the software package for statistical analysis SPSS – 17.0 Windows version (Chicago, Il, USA).

**Results and discussions**

The results obtained after these investigations of these three groups were reported as averages ± SEM. They’re represented in table 1.

In our case, having a patients and groups spread out equally, the Tukey test was used. After comparing groups A, B and C, the statistical values presented in detail in tables 2 and 3 were obtained.

To determine the correlation coefficient between variables and to analyze the effect of a variable on another the Pearson(r) test was used. Significant and positive correlations were found between fasting glucose salivary calcium concentration and HbA1c, graphs 1, 2 and 3 represent these correlations.

Numerous published literatures describe sialosis as being specific to diabetes. This is represented by degenerative modifications, by an increase in volume of salivary glands due to the infiltration of fat but also modifications of acini glands with secretion affects. These complex modifications are neuroendocrine controlled [21, 22].

On the other hand, other authors believe that the decrease in salivary secretion for patients with type II diabetes mellitus can be attributed to increased diuresis, which causes the reduction of extra cellular fluid and therefore decreases the volume of saliva [22-24].

Decreased saliva, appreciated by us measuring the unstimulated salivary flow, is more evident for patients who fail to manage to maintain control of glycemic factors. Numerous studies have confirmed for these cases an

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**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (healthy)</th>
<th>Group B (HbA1c&lt;8%)</th>
<th>Group C (HbA1c≥8%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>86.60 ± 1.514</td>
<td>118.51 ± 2.057</td>
<td>202.65 ± 9.910</td>
</tr>
<tr>
<td>Hb A1c (%)</td>
<td>5.43± 0.108</td>
<td>7.07±0.071</td>
<td>12.516±0.500</td>
</tr>
<tr>
<td>Salivary pH</td>
<td>7.051 ± 0.067</td>
<td>6.162 ± 0.145</td>
<td>6.039 ± 0.152</td>
</tr>
<tr>
<td>Salivary calcium (mg/100ml)</td>
<td>9.893 ± 0.233</td>
<td>5.028 ± 0.287</td>
<td>5.261 ± 0.307</td>
</tr>
<tr>
<td>Flow rate (ml/min)</td>
<td>0.452± 0.028</td>
<td>0.279± 0.021</td>
<td>0.293± 0.024</td>
</tr>
<tr>
<td>DMF-T-1</td>
<td>20.95 ± 0.730</td>
<td>25.77 ± 0.737</td>
<td>25.79 ± 0.524</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>(I)group</th>
<th>(J)group</th>
<th>Salivary pH</th>
<th>Salivary Calcium</th>
<th>Flow rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>Mean difference</td>
<td>Std. Error</td>
<td>Sig.</td>
</tr>
<tr>
<td>0.53912</td>
<td>0.18091</td>
<td>0.009</td>
<td>3.36256</td>
<td>0.18091</td>
</tr>
<tr>
<td>C</td>
<td>B</td>
<td>0.91246</td>
<td>0.18091</td>
<td>0.000</td>
</tr>
<tr>
<td>B</td>
<td>C</td>
<td>-0.53912</td>
<td>0.18091</td>
<td>0.009</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td>0.47333</td>
<td>0.18091</td>
<td>0.026</td>
</tr>
<tr>
<td>C</td>
<td>A</td>
<td>-0.18126</td>
<td>0.18091</td>
<td>0.000</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>0.07303</td>
<td>0.18091</td>
<td>0.026</td>
</tr>
</tbody>
</table>

*the correlation is significant at level 0.05*
increase in oral cavity inflammation. These may show such signs as: ulcerations, cheilitis, tongue fissures, damaged dentures, periodontal infections [4, 10, 13].

The presence of salivary calcium is the key in the process of demineralization/re-mineralization in the oral cavity. Mineral ions present in blood serum is in a permanent change with the saliva secreted by the salivary glands. This washes dental plaque and hard and soft tissues acting as a reservoir of calcium to maintain physiological salivary buffer limits [4].

For people with hyperglycemia, protective factor of saliva’s pH, salivary flow and adequate calcium levels can be strongly disturbed [21, 25].

We found that there was a statistically significant difference in our study (p < 0.0001) between the glycemic levels of patients in group A 86.60 ± 1.514 which have an index DMF-T = 20.95 ± 0.730 compared to group C (diabetes with uncontrolled glycemic levels HbAc ≥ 8) 292.65 ± 9.910 with index DMFT = 25.79 ± 0.524.

These results suggest the processes of demineralization/re-mineralization, in the oral cavity, can be controlled by suitable inorganic ions in saliva concentration. These results are consistent with other studies that confirm that people with an uncontrolled blood glucose level present a long-term risk for dental caries is higher than in patients with a controlled blood glucose level [4, 6, 13, 23].

Analyzing Tukey test results it is found that the difference in pH average between healthy patients respectively diabetics is 1.01246 significantly to P < 0.001 lower than the critical threshold 0.05, which allows us to have a error risk level of less than 5 % as patients in group C (având HbAc1 > 8%) have a more acidic pH level than patients in Group A (clinically healthy patients).

Similarly, the difference in salivary pH levels averages between group B and C is 0.47333 significantly for P = 0.026. Which P (significance) is smaller than the critical threshold of 0.05, subjects in group C have more acidic pH level than subjects in group B.

On the other hand, we cannot entirely attribute the cario-protection effects only to pH levels and salivary calcium concentration. Besides these components, along with oral biocenosis there are also a number of protective factors against the demineralization caused by organic acids. Amongst these, the salivary film that covers the teeth also plays an important role, which is composed of calcium ions bound by protein. This film can function for a short period of time, as a selective permeable membrane which is implicated in reducing the solubility of hard dental structures. [18, 24-27].

<table>
<thead>
<tr>
<th>(I)group- (J)group</th>
<th>Fasting glucose</th>
<th>HbA1c</th>
<th>DMF-T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean difference</td>
<td>Std. Error</td>
<td>Sig.</td>
</tr>
<tr>
<td>A B</td>
<td>-31.912</td>
<td>8.356</td>
<td>0.001</td>
</tr>
<tr>
<td>A C</td>
<td>-206.053</td>
<td>8.356</td>
<td>0.000</td>
</tr>
<tr>
<td>B C</td>
<td>31.912</td>
<td>8.356</td>
<td>0.001</td>
</tr>
<tr>
<td>A A</td>
<td>-174.140</td>
<td>8.356</td>
<td>0.000</td>
</tr>
<tr>
<td>C A</td>
<td>206.053</td>
<td>8.356</td>
<td>0.000</td>
</tr>
<tr>
<td>B B</td>
<td>174.140</td>
<td>8.356</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* the correlation is significant at level 0.05

Fig. 1. Pearson Correlation between fasting glucose and Salivary calcium is 0.543 (significant at the 0.01 level).

Fig. 2. Pearson Correlation between fasting glucose and HbA1c is 0.846 (significant at the 0.01 level).

Fig. 3. Pearson Correlation between HbA1c and Salivary calcium is 0.518 (significant at the 0.01 level).
Conclusions

Demineralization/re-mineralization processes in the oral cavity which determine carious lesions are strongly influenced by the salivary factor. The protective role of saliva is due to the salivary flow rate, the pH level and salivary calcium concentration.

These protective components are disrupted in patients with type II diabetes mellitus, especially for those with uncontrolled blood glucose values, highlighted by given glycosylated hemoglobin.

Through a long-term glycemic control, it is possible to maintain DMF-T index at a low level. The decrease of salivary calcium concentration may be associated with the initiation and progress of new carious lesions.

References

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