Association between dental-oral health in young adults and salivary glutathione, lipid peroxidation and sialic acid levels and carbonic anhydrase activity

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The aim of the present study was to evaluate the relationship between salivary oxidative stress and dental-oral health. Healthy young adults, matched for gender and age, with (N = 21, 10 men, mean age: 20.3 ± 1 years) and without (N = 16, 8 men, mean age: 21.2 ± 1.8 years) caries were included in this study. The World Health Organization (WHO) caries diagnostic criteria were used for determining the decayed, missing, filled teeth (DMFT) index. The oral hygiene and gingival status were assessed using the simplified oral hygiene index and gingival index, respectively. Unstimulated salivary total protein, glutathione (GSH), lipid peroxidation and total sialic acid levels, carbonic anhydrase activity, and salivary buffering capacity were determined by standard methods. Furthermore, salivary pH was measured with pH paper and salivary flow rate was calculated. Simplified oral hygiene index and gingival index were not significantly different between groups but DMFT scores were significant (P < 0.01). Only, GSH values were significantly different (P < 0.05) between groups (2.2 and 1.6 mg/g protein in young adults without caries and with caries, respectively). There was a significant negative correlation between DMFT and GSH (r = -0.391; P < 0.05; Pearson’s correlation coefficient). Our results suggest that there is an association between caries history and salivary GSH levels.

Key words: Glutathione; Lipid peroxidation; Sialic acid; Carbonic anhydrase; Dental-oral health

Received June 3, 2008. Accepted October 2, 2008
SFR was calculated as mL/min. Saliva samples were stored at -20°C until use.

After thawing at 4°C for 1 h, the samples were centrifuged and supernatants were used. Salivary pH was directly measured with pH paper (Merck Neutrolit-5.5-9.0, Darmstadt, Germany) and salivary buffering capacity was measured by the Ericsson method. No differences were found between groups for salivary pH and SFR.

One experienced dentist examined all subjects for their adherence to inclusion criteria. World Health Organization diagnostic criteria were used for determining the decayed, missing, filled teeth (DMFT) index. The oral hygiene and gingival status were assessed using the simplified oral hygiene index (OHI-S) and gingival index (GI) (1). OHI-S and GI were not significantly different between groups but DMFT scores were significantly different (P < 0.01; Table 1).

Good oral hygiene may contribute to saliva composition as an oxidative stress-decreasing factor in dental diseases (2). Limited data are available about the effect of oral hygiene on salivary parameters in subjects with caries. Dental caries is a multifactorial disease. Diet, host (saliva and tooth), bacteria, time and personal factors (oral hygiene) are responsible for the development of dental caries. The first line of defense against dental caries is saliva. The composition and physiology of saliva warrants thorough investigation because it clearly influences oral health (3).

The antioxidant defense system of saliva has several components. One of these antioxidants is GSH, a tripeptide containing an SH group. It has been reported that the levels of GSH in saliva are altered by different factors (4,5) and that salivary GSH levels decrease in periodontal diseases (2,5). Interestingly, salivary GSH levels and the relevance of this to caries protection have not yet been investigated. In the present study, salivary GSH levels, which were determined by the method of Beutler (6), were significantly lower in subjects with caries compared to subjects without caries (Table 1). Moreover, there was a significant negative correlation (r = -0.391; P < 0.05; Pearson’s correlation coefficient) between GSH and DMFT index. This may be attributed to an antioxidant effect of salivary GSH against caries formation.

Table 1. Dental and salivary parameters for young adults with and without caries.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Caries-free group (DMFT = 0; N = 16)</th>
<th>Caries group (DMFT = 5.6; N = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral hygiene index</td>
<td>0.14 ± 0.008</td>
<td>0.15 ± 0.011</td>
</tr>
<tr>
<td>Gingival index</td>
<td>0.017 ± 0.008</td>
<td>0.02 ± 0.009</td>
</tr>
<tr>
<td>Salivary pH</td>
<td>7.1 ± 0.4</td>
<td>7.2 ± 0.37</td>
</tr>
<tr>
<td>Salivary flow rate (mL/min)</td>
<td>0.51 ± 0.22</td>
<td>0.51 ± 0.25</td>
</tr>
<tr>
<td>Salivary buffering capacity</td>
<td>1.6 ± 0.3</td>
<td>1.5 ± 0.31</td>
</tr>
<tr>
<td>Salivary total protein (mg/dL)</td>
<td>128.4 ± 41</td>
<td>132.6 ± 40</td>
</tr>
<tr>
<td>Glutathione (mg/g protein)</td>
<td>2.2 ± 0.8</td>
<td>1.6 ± 0.75*</td>
</tr>
<tr>
<td>Lipid peroxidation (µmol MDA/g protein)</td>
<td>0.33 ± 0.28</td>
<td>0.3 ± 0.15</td>
</tr>
<tr>
<td>Total sialic acid (mg/g protein)</td>
<td>36.3 ± 12.8</td>
<td>36 ± 14.56</td>
</tr>
<tr>
<td>Carbonic anhydrase activity (units/g protein)</td>
<td>26.8 ± 6.8</td>
<td>27.2 ± 11.03</td>
</tr>
</tbody>
</table>

Correlation analysis variables Correlation coefficient (r)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione - DMFT</td>
<td>-0.391 (P &lt; 0.05)</td>
</tr>
<tr>
<td>Total protein - carbonic anhydrase</td>
<td>-0.527 (P &lt; 0.01)</td>
</tr>
<tr>
<td>Lipid peroxidation - total sialic acid</td>
<td>0.338 (P &lt; 0.05)</td>
</tr>
</tbody>
</table>

Data are reported as means ± standard deviation. DMFT = decayed, missing, filled teeth; MDA = malondialdehyde. *P < 0.05, significantly different from caries-free group (Student t-test between groups and Pearson’s correlation analysis).
in the morning before saliva collection. The reason why we did not find significant differences in salivary LPO levels between groups may be due to tooth brushing.

Salivary carbonic anhydrase (CA VI, EC.4.2.1.1, a zinc metalloenzyme) is the only known secreted isoenzyme of the CA family, which has been detected in the saliva secreted by the serous acinar cells of mammalian parotid and submandibular glands. It catalyzes the reaction by which bicarbonate ions neutralize the acids formed by plaque bacteria (10). There is evidence to suggest that salivary CA is a multifunctional enzyme, which affects taste bud growth, protecting the teeth from caries, and as an anti-inflammatory agent (10,11). Low salivary CA VI concentration is associated with the increased prevalence of caries, and a negative correlation between CA VI concentration and DMFT index in individuals with poor oral hygiene has been reported by Kivela et al. (10). In the present study, no significant difference in CA VI activity was detected between groups and a non-significant negative correlation was found between DMFT index and salivary CA activity in the caries group ($r = -0.246; P > 0.05$; Pearson’s correlation coefficient). It is possible that we did not find a significant difference in this parameter between young adults with and without caries due to good oral hygiene and gingival health of our subjects.

It has been reported that subjects with caries and without caries have different salivary protein profiles (12). Although no significant changes were found in the salivary total protein level and CA VI activity between groups in the present study, a significant negative correlation ($r = -0.527; P < 0.01$; Pearson’s correlation coefficient) was found between salivary total protein and CA activity. This indicates that protective proteins decreased in the saliva of subjects with caries. Total protein levels and CA activity were determined by the methods of Lowry et al. (13) and Verpoorte et al. (14), respectively.

Most of the salivary proteins are glycoproteins. SA is one of the terminal residues of salivary glycoproteins. It is an important structural component of salivary glycoproteins, enhancing bacterial aggregation as well as participating in the formation of the acquired pellicle and dental plaque (15). Salivary SA levels are affected by oral diseases (16,17). More recently, it has been reported that salivary SA increased with salivary oxidative stress (18).

Saliva from subjects without caries has been found to promote aggregation more strongly than saliva from those with caries. Saliva from subjects with caries likewise was more effective in causing adherence (15).

We did not detect significant differences in saliva SA levels between groups, as determined by the method of Warren (19). Salivary total SA levels were higher in subjects with caries in only one study (16). We are not able to compare this observation with our results because DMFT, GI and OHI-S scores were not reported.

A recent study suggests that bovine submaxillary mucin has hydroxyl radical scavenging ability, and the SA in mucin is an essential moiety to scavenge hydroxyl radicals and mucin synthesis is induced by oxidative stress (20). Though no significant differences were found either in the salivary total SA or LPO levels between groups in the present study, there was a significant positive correlation ($r = 0.338; P < 0.05$; Pearson’s correlation coefficient) between these parameters. A possible explanation for this relationship is that LPO induces mucin synthesis and thus total SA in saliva. However, it is also well known that oxidative stress causes hydrolysis and liberation of terminal SA of glycoproteins (18). If terminal SA is released by LPO, what is the effect of this rupture on bacterial adhesion and aggregation? These are questions waiting to be answered and the underlying mechanisms involved at the molecular basis need to be further explored.

References


