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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

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In this study, the relationship between dental caries and glutathione (GSH) as an important antioxidant, lipid peroxidation (LPO) as an indicator of oxidative damage of oral tissues, sialic acid (SA) as a mediator for bacterial adhesion, carbonic anhydrase (CA) activity as a key enzyme for oral physiology and other salivary parameters such as salivary buffering capacity, pH and saliva flow rate (SFR) were evaluated in healthy young adults with and without caries.

The present study was designed in accordance with the guidelines issued in the Declaration of Helsinki and approved by the local Ethical Committee. Written informed consent was obtained from all participants. A total of 37 healthy young adults with (N = 21, 10 men) and without (N = 16, 8 men) caries, who are students at the Dental Faculty, were included in this study. Their ages were between 19 and 25 years and the mean were 21.2 and 20.3 years for caries-free and with caries subjects, respectively ($P > 0.05$). All subjects were instructed to refrain from smoking, eating and drinking for 12 h prior to saliva collection and to brush their teeth in the morning. Fasting unstimulated whole saliva samples were always collected between 8:30 and 11:00 h. Before saliva collection, the mouth was rinsed with distilled water. Subsequently, saliva was allowed to accumulate on the floor of the mouth and the subjects were instructed to spit into a test tube. Each saliva collection period was 10-min long. Immediately after collection, saliva volume was measured and the

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.

LPO, which is one of indicators of oxidant damage, causes degeneration of the cell membrane of oral tissue during inflammatory oral diseases (2,4). It has been suggested that the impairment of oxidant-antioxidant balance in saliva causes oral diseases (4). In the present study, LPO levels, which were determined by the method of Ledwozyw et al. (7), were similar for both the caries and caries-free groups. In the literature, salivary LPO levels have been reported to be higher in individuals with poor oral hygiene compared to subjects with adequate oral hygiene (2,5). Rai et al. (8) determined the LPO product malonaldehyde (MDA) in some oral diseases, such as leukoplakia, oral submucous fibrosis, candidiasis, dental caries, and oral cancer, and in healthy subjects. They reported that there were no differences in salivary MDA levels in a caries group compared to control. However, increased salivary LPO levels in periodontal and some systemic diseases, such as diabetes, osteoporosis, etc., that were related with oxidative damage, are well known. Hodosy and Celec (9) have investigated the effects of daily dynamics, tooth-brushing and ascorbic acid administration on salivary thiobarbituric acid reacting substance (TBARS) levels. They reported that tooth-brushing decreases salivary TBARS. In the above-mentioned studies (8,9), since DMFT and oral hygiene status of the subjects were not determined, we are not able to compare them to our results. However, in the present study, the subjects routinely brushed their teeth and they were also reminded to brush their teeth

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

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

Correlation analysis variables	Correlation coefficient (r)
Glutathione - DMFT	-0.391 ($P < 0.05$)
Total protein - carbonic anhydrase	-0.527 ($P < 0.01$)
Lipid peroxidation - total sialic acid	0.338 ($P < 0.05$)

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) found between salivary total protein and CA activity may indicate 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.

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