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Original Article

Salivary cortisol and total antioxidant capacity levels of children with untreated dental caries

Gul Keskin[1], Zubeyde Ucar Gundogar[1], Hakan Ozdogru[1], Hasan Ulusal[2], Hasan Gundogar[3]

[1]Department of Pediatric Dentistry, Faculty of Dentistry, Gaziantep University, Gaziantep, Turkey
[2]Department of Clinical Biochemistry, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey
[3]Department of Periodontology, Faculty of Dentistry, Gaziantep University, Gaziantep, Turkey

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Abstract

Aim: The study aimed to evaluate the relationship between the caries frequency, which was determined using different caries indexes and biological parameters such as salivary cortisol and total antioxidant capacity (TAC).

Materials and Methods: 140 patients between the ages of 5-13 without systemic disease were included in the study. Saliva samples were collected from patients whose DMFT (dmft) and PUFA (pufa) indexes were recorded, and these samples were centrifuged at 5,000 rpm for 10 minutes. All samples were stored at -20 °C until measurement. Saliva cortisol and TAC levels were measured using the ELISA kit. All data were analyzed with SPSS v.22.

Results: The mean DMFT/dmft and PUFA/pufa values were estimated as 6.72±4.37, 1.39±2.08, respectively. There was no significant correlation between caries indexes and biochemical parameters.However, there was a correlation between TAC and cortisol levels in saliva.

Conclusion: Within the limitations of this study, it was determined that high risk of caries in patients could not affect salivary cortisol and TAC levels; however, there was a positive correlation between salivary cortisol and TAC levels. Further studies are needed to better understand the background of the pathophysiological changes in these biochemical parameters and their relationship with caries.

Keywords: Cortisol; dental caries; saliva; total antioxidant capacity

INTRODUCTION

Dental caries, which is called dissolution of dental structures due to prolonged periods of low pH, is a common, infectious, and inflammatory disease (1). Many factors affect the onset and progression of the disease (2). Genetic and environmental determinants related to disease risk and resistance, and they show dynamic effects on the disease process (3). Cariogenic bacteria, poor dietary habits, and the absence of saliva are among the environmental factors that causing dental caries (4).Additionally, it has been suggested that stress may be a potential etiologic factor for caries formation (5,6). Alterations in saliva composition and saliva flow rate due to stress may be a factor explaining the relationship between caries and stress (7). Salivary secretion may decrease in recurrent chronic stress conditions depends on affecting the Autonomic nervous system, which regulates saliva secretion. Also, it may increase the risk of dental caries (8).

Cortisol is a hormone secreted by the hypothalamus-pituitary-adrenal axis. Moreover, It is accepted as a stress biomarker in both adults and children. Saliva cortisol levels are accepted as a reliable, accurate, and noninvasive stress measurement method (9). It has also been claimed that cortisol is released by the system to struggle the effect of chronic inflammatory conditions(10). Based on the hypothesis, dental caries is an inflammatory disease and stress is a potential etiological factor for dental caries, the increased number of caries can be associated with increased saliva cortisol level (11-13).

It has also been recommended that continuous stimulation of the hypothalamic-pituitary-adrenal axis due to chronic inflammation and prolonged stress causes oxidative damage (14,15). Oxidative stress (OS) occurs as a result of an imbalance in favor of oxidants among oxidants and antioxidants, and it is believed to play a role in various adverse processes in an organism (16). Recently, it has been claimed that imbalances in the level of free radicals, reactive oxygen species, and saliva antioxidants can play an essential role in the onset and development of dental caries (17). Saliva antioxidants can interact with free radicals or reactive oxygen species that cause oxidative

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Corresponding Author: Zubeyde Ucar Gundogar, Department of Pediatric Dentistry, Faculty of Dentistry, Gaziantep University, Gaziantep, Turkey **E-mail:** zubeydeucargundogar@hotmail.com

stress to limit the progression of a disease. The combined ability of a group of enzymes, including saliva antioxidants, saliva peroxides, saliva uric acid, and a few small enzymes, is often referred to as saliva's total antioxidant capacity (TAC) (12).

There are studies that investigated the relationship between caries-cortisol and caries-TAC in the literature review. However, no study evaluating the correlation of caries, cortisol and TAC parameters was found.In this study, we aimed to evaluate the relationship between the caries frequency, which was determined using different caries indexes and biological parameters such as salivary cortisol and TAC. The hypothesis determined for the study in the light of this information is that the caries indexes and salivary cortisol and TAC will correlate with each other.

MATERIALS and METHODS

Subjects

Ethics committee approval was obtained from the Gaziantep University Clinical Research Ethics Committee (2019/154). The study included 140 pediatric patients between 5-13 years, who applied to the Department of Pediatric Dentistry for examination.Patients with systemic and congenital disorders, receiving chemotherapy and radiotherapy, using drugs that affect saliva such as antidepressants, corticosteroids, and giving insufficient saliva samples were excluded from the study. Before the procedure, children and parents were informed about the study, and consent forms were obtained.

Determination of DMFT/dmft ve PUFA/pufa indexes

The clinical examination of the children was carried out by a single researcher. Dental examination was carried out under natural light after the air drying of the teeth and with the help of a dental mirror and CPI probe.

DMFT index; All teeth were evaluated according to the criteria recommended by the World Health Organization (WHO) using the “dmft” and “DMFT” index, respectively, for primary and permanent teeth.When the brushed and air-sprayed surfaces of the tooth gave a 'chalky-white' appearance, these were defined as cavitation-free lesions. When at least one of the surface cavitation or white spot lesions showed clinical signs, teeth were accepted as caries. Bitewing radiographs were taken to detect proximal caries.

PUFA index; It was recorded separately from the DMFT index and scored according to the following codes and criteria:Only one point is given per tooth. If the primary tooth and permanent tooth are present, and both indicate the stages of odontogenic infections, both teeth are scored. Capital letters are used for permanent teeth, and small letters are used for primary teeth. The PUFA score per person is calculated in the same cumulative manner as dmft and represents the number of teeth that meet the PUFA diagnostic criteria. For permanent teeth and primary teeth, PUFA is recorded separately. Therefore, the score for a person ranges from 0 to 20 pufa for the primary tooth and 0 to 32 PUFA for the permanent tooth.

Collection of saliva samples, cortisol, and TAC analysis

The unstimulated saliva samples of the patients participating in the study were collected between 9 and 12 o'clock in the morning, after one hour of the children brushed their teeth by one researcher.As the samples were collected, the children were seated with their heads slightly down. Saliva was allowed to accumulate in the mouth for 2 minutes. Later, they were asked to spit the saliva collected in their mouths into the pet cups provided. Besides, they were asked not to perform mouth, muscle, tongue, and lip movements during the procedure to increase the amount of saliva, and not to swallow. The collected saliva was transferred to Eppendorf microtubes via volume samplers and centrifuged (NF 200 centrifuge machine) at 5000 rpm for 10 minutes. All samples were stored at -20 C0 after centrifugation, and cortisol and TAC levels were analyzed using the ELISA kit (DRG Salivary Cortisol ELISA; DRG International, Inc., USA) according to manufacturer's instructions. While cortisol levels were determined in the picogram/microliter, TAC was calculated in micromoltroxolecuvalent/liter.

Statistical analysis

According to previously published study (9), for 80% power (1-ß), the present study is needed 141 individuals in order to detect a relationship between saliva cortisol level and clinical parameters.Firstly, the normality of numerical data was tested with the Shapiro Wilk test. Relations between numerical variables were tested with the Spearman Rank test, and correlation coefficient values were evaluated. For the descriptive statistics, mean ± std. Deviation, minimum, and maximum values are given for each data. SPSS v22.0 (for Mac) package program was used in the statistical analysis.

RESULTS

One hundred forty children-74 female, 66 male-participated in our study. The mean age range was 8.67 ± 2.18. The caries index data obtained from the patients and the mean and standard deviation values of the saliva biomarkers are given in Table 1. While DMFT/dmft data is in the range of 0-20(6.72 ± 4.37), PUFA/pufa data is in the range of 0-11(1.39 ± 2.08). The mean values of Cortisol and TAC were 6,209 ± 4,362 and 0,284 ± 0,22, respectively.

Table 1. Descriptive values of caries index and biochemical parameters in saliva

	Min	Max	Mean (Standart Deviation)
DMFT/dmft	0	20	6.72 (4.37)
PUFA/pufa	0	11	1.39 (2.08)
Cortisol (pg/µl)	1.021	35.101	6.209 (4.362)
TAC (mmolTrolox equivalent/l)	0.055	1.236	0.284 (0.22)

According to Spearman correlation analysis, there was no correlation between caries indices and cortisol and TAC, but there was a correlation between cortisol and TAC (Table 2).

Table 2. Correlation coefficient of clinical and biochemical data

Correlations	DMFT/dmft	PUFA/pufa	Cortisol	TAC
DMFT/dmft	1000	.494**	-.070	.197
PUFA/pufa	.494**	1.000	-.012	.056
Cortisol	-.059	.074	1.000	.231**
TAC	.025	-.058	.231**	1.000

DISCUSSION

Salivary cortisol level is routinely biomarker of psychological stress and related mental or physical disease (18). Cortisol levels can be analyzed in blood, urine, and saliva. Collecting saliva samples is a non-invasive method compared to blood collection and, therefore, does not create stress and potentially higher cortisol (19). Although the saliva cortisol concentration constitutes only about 50-60% of the plasma cortisol concentration, a large number of publications have shown that saliva cortisol is an accurate measure of adrenocortical function as the stress index. It is also known that serum cortisol is transferred to saliva quickly (within 5 min) and is not affected by saliva flow rate (11). In the determination of antioxidant defense parameters, it has been suggested that unstimulated saliva should be preferred because TAC is higher in unstimulated saliva (20). Therefore, in this study, unstimulated saliva samples were used to determine both cortisol and TAC.

DMFT/dmft is an index system used worldwide to collect data about dental caries, but this cumulative index does not provide information about the clinical consequences of untreated dental caries such as pulp affected, abscess formation (21). The PUFA/pufa index has been defined to determine the severity and prevalence of oral conditions caused by untreated dental caries by Monse et al.(22) Since the PUFA/pufaindex reflected thedental caries byan inflammatory aspect, the correlation between this index with cortisol and TAC is thought to be stronger. However, when the data obtained from the present study are evaluated, there was no correlation between caries frequency and biochemical parameters. In this case, the hypothesis can be rejected.

The relationship between caries and cortisol is explained in two ways. The first is that under repeated chronic stress conditions, salivary secretion will decrease, and high cortisol reactivity against stress can increase the risk of dental caries by undermining local protective defenses and micro anatomic structures (8,13). Another is; the pain and discomfort that occurs in children with common infection and dental caries will cause stress, and this will increase the salivary cortisol levels(11). Several studies have reported a correlation between increased caries and saliva cortisol levels (11,12). However, Kambalimath et al. (23) reported that there was no statistically significant difference in the salivary cortisol levels of children with and without caries before treatment, after oral prophylaxis or fluoride application.The data obtained from this study are similar to the present study. It has also been reported that saliva cortisol levels may remain normal in children under chronic stress (24).

Increased TAC levels with increasing frequency of caries have been reportedin several studies (2,17,25-32). The characteristic feature of these studies is that they compare children with severe early childhood caries or rampant caries to children without caries.In these severe forms of caries, an increase in TAC may have occurred due to increased inflammatory response. In the present study, DMFT (dmft) / PUFA (pufa) values of children were varied between 0 and 20/11. This wide range of distribution may be the reason for the lack of a statistically significant difference between the caries indexes and saliva TAC. Evaluating a specific group can lead to more meaningful results.

In the present study, a strong correlation was found between the saliva cortisol and TAC levels in the samples obtained from patients. It has been suggested that continuous stimulation of the hypothalamic-pituitary-adrenal axis due to prolonged stress can cause oxidative damage(14). The result obtained may be related to this situation. Also, the correlation between this cortisol and TAC obtained from the study is similar to the literature studies (16,33).

CONCLUSION

Within the limitations of this study, it was found that there was a strong correlation between salivary cortisol and TAC levels, but there was no significant relationship between caries indexes and saliva parameters. These results do not support the view that metabolic changes due to stress may be an etiological factor in terms of dental caries.In this case, it seems more likely that cortisol and oxidative stress tend to increase in the presence of severe pain and infection due to dental or orofacial pathologies.However, different results reported in the literature in this regard are insufficient to explain the relationship between caries and saliva biomarkers. Therefore, additional studies on the subject are needed.

Conflict of interest: The authors declare that they have no competing interest.

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