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Original Research

Effect of Past and Current Dental Treatment on Children's Dental Anxiety Status and Oxidative Stress Biomarkers: A Pilot Study

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ABSTRACT

Objective: This study aimed to detect dental anxiety caused by past and current dental treatment in children using saliva biomarkers and to investigate the correlation between these markers and psychometric analysis.

Methods: A total of 43 children aged 6–13 years were recruited for this study. The participants were divided into two groups: those with dental treatment experience (G1) and those who presented to the dentist for the first time (G2). Anxiety scale data and saliva samples were obtained. Oxidative stress (OS) markers, namely, total antioxidant capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI), were analyzed from the saliva samples. After current treatments such as fluoride application or restorative procedures, anxiety scale data, and saliva samples were obtained.

Results: TAC and OSI values were higher in G1 than in G2 at baseline ($p<0.05$), and anxiety scale values were similar in both groups ($p>0.05$). After the current treatment, a significant decrease in TAC and OSI values was found in G1 ($p<0.05$), but no significant difference was observed in G2. Anxiety scale values were significantly decreased after treatment in G2 ($p<0.05$). Although a strong correlation was found between baseline TAC, TOS, OSI, and post-treatment TAC and OSI values ($p<0.001$), no correlation was noted between OS biomarkers and anxiety scale values ($p>0.05$).

Conclusion: These results suggested a potential relationship between anxiety and OS biomarkers, but additional studies are needed to understand the relationship between dental anxiety and pathophysiological changes in OS biomarkers.

Keywords: Dental anxiety, oxidative stress, dental treatment, anxiety scale

INTRODUCTION

Dental anxiety is a state of constant and extreme fear of dental stimulants and procedures. If a patient has anxiety, the frequency of dental caries may increase due to the decrease in the frequency of visit to the dentist (1). Although its etiology is unclear, three mechanisms were proposed for the development of dental anxiety: indirect development via vicarious learning/modeling, direct development through direct conditioning, or a person's inherent personality traits (2). In the literature, the term state anxiety, which means "anxiety at the present moment," has also been defined (3). While dental anxiety involves the individual emotional state for dental treatment shaped by experiences or modeling, state anxiety can reflect a situation-specific emotional experience for a particular dental procedure, and studies have shown that it can fluctuate at different treatment stages (4). Thus, treatment planning should be directed in a flexible and patient-based manner in stages, and treatment should be started with the least fearful, painless, and nontraumatic techniques, especially in children with anxiety and no dental treatment experience (5).

Four different measurement techniques are recommended for the assessment of dental anxiety in children. These include projective tests, psychometric techniques, various scales used to examine and evaluate a child's behavior during a dental visit, and physiological measurements (6). It is difficult to talk and agree with younger patients. Thus, studies have recommended the use of simple and visual projective methods that do not require verbal communication and are applicable in this patient group (7, 8). In psychometric techniques that include self-report measurements, a dental anxiety score is obtained by asking children directly about their concerns with the help of a scoring scale. This method is usually performed through a survey or an interview (9). One of the most widely used psychometric techniques in children is the modified child dental anxiety scale (MCDAS) (10). This scale has been modified for easier application in younger

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children. The MCDAS faces version ($MCDAS_f$), which was created by adding a cartoon faces rating scale to the original numerical form, is a versatile scale that can be applied to children aged 5–12 and with limited cognitive function (11).

In addition to psychological changes in dental anxiety observed in children, dental anxiety may lead to the secretion of some mediators, that is, biochemical changes, as a result of stress or mood disorders affecting the adrenal system. Studies have suggested that continuous stimulation of the hypothalamus–pituitary–adrenal axis due to prolonged stress causes oxidative damage (12, 13). Oxidative stress (OS) results from increased production of reactive oxygen species and reduced concentration/activity of antioxidants responsible for their neutralization (14). Imbalances in the levels of free radicals and antioxidant-reactive oxygen species can play an important role in the onset and development of various inflammatory oral pathologies (15). Total antioxidant capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) are biochemical markers that can be used to measure OS. TAC is the total capacity of both enzymatic and non-enzymatic antioxidants, TOS provides an assessment of the content of all oxidants in a sample, and OSI shows the relationship between antioxidant mechanisms and oxidant concentrations (16). Evaluation data of these OS biomarkers in the saliva have also been used to identify or describe anxiety-causing events such as dental treatment in children (17).

To the best of our knowledge, no study has evaluated the relationship between dental experience-based anxiety and OS biomarkers. In the light of this, this pilot study aimed to detect dental anxiety caused by past and current dental treatment in children by using saliva biomarkers and to investigate the correlation between these markers and psychometric analysis. The study hypotheses were as follows:

- Increased OS biomarker values would be obtained in children with dental treatment experience compared with children without any dental treatment experience.
- There would be a significant decrease in OS biomarkers in both groups after the procedure.
- The anxiety scale data obtained at baseline and after treatment would be correlated with OS biomarkers.

METHODS

Participants

In this study, ethics committee approval was obtained from the Gaziantep University/Turkey Clinical Research Ethics Committee (2019/153). The study included 43 patients aged 6–13 years.

Main Points:

- High OS biomarker values decreased significantly in patients who had undergone surgical treatment.
- Decreased anxiety scale values were observed in each group, especially in G2, after the treatment.
- A strong correlation was found between OS biomarkers at baseline and after treatment.

Patients with systemic and congenital disorders, receiving chemotherapy and radiotherapy, taking medications that affect the saliva such as antidepressants and 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. The participants were divided into two groups: those with dental treatment experience including surgical procedures (G1, n=18) and those who visited a dentist for the first time (G2, n=25). A questionnaire including demographic data and $MCDAS_f$ data was administered to the participants.

Anxiety Scale

The $MCDAS_f$ questionnaire contains eight questions about dental procedures that can trigger stress such as local anesthesia, sedation, general anesthesia, and tooth extraction, and five faces indicating a visual emotional state for each question. Scores range from 8 to 40: the happiest and unhappiest faces were rated 1 and 5, respectively. The Turkish version of this scale was filled out by the dentist in an interview, in line with the answers given by the patient.

Collection of Saliva Samples and TAC, TOS, and OSI Analyses

The unstimulated saliva samples of the participants were collected by a researcher between 9 and 12 o'clock in the morning, which was 1 hour after the participants brushed their teeth. During sample collection, the children were seated with their heads slightly down and their saliva was allowed to accumulate in the mouth for 2 min. Later, they were asked to spit the collected saliva into the pet cups. In addition, they were asked not to move their mouth, buccinator muscles, tongue, and lip during the procedure to increase the amount of saliva, and not to swallow them. The collected saliva was transferred to Eppendorf microtubes through volume samples and centrifuged (NF 200 centrifuge machine) at 5000 rpm for 10 min. All samples were stored at −20°C after centrifugation, and TAC, TOS, OSI levels were analyzed using an enzyme-linked immunosorbent assay kit (DRG Salivary Cortisol ELISA; DRG International, Inc., USA) according to manufacturer's instructions.

After baseline data were obtained, fluoride application or restorative procedures were performed, and then anxiety scale data and saliva samples were obtained.

Statistical Analysis

The compatibility of all numerical data to normal distribution was tested by the Shapiro–Wilk test. Mann–Whitney U test was used to compare non-normally distributed variables between groups. Analysis of variance and Tukey comparison tests were used to compare normally distributed numerical data, and Kruskal–Wallis and allpairwise tests were used to compare non-normally distributed data. Relationships between numerical variables were tested with Spearman rank correlation coefficient. The average statistics are presented as mean ± standard deviation for introductory statistics. SPSS Statistics v22.0 (IBM SPSS Corp.; Armonk, NY, USA) for Mac was used in the analysis. $p<0.05$ was considered significant.

Table 1. Mean Oxidative stress marker values and anxiety scale data at baseline and after treatment

	TAC		TOS		OSI		Scale	
	B	AT	B	AT	B	AT	B	AT
G1 (n=18)	7.37±2.63*	5.44±2.39*$^{\delta}$	4.54±2.64	5.07±1.91	2.16±1.48*	1.18±0.57$^{\delta}$	19.32±5.53	18.17±5.3
G2 (n=25)	3.08±1.89	3.68±2.19	5.51±2.44	5.24±2.03	0.76±0.77	0.89±0.7	20.17±5.26	17.09±4.9$^{\delta}$

*Statistical significance between parameters between groups
$^{\delta}$Statistical significance relative to baseline.
TAC, total antioxidant capacity; TOS, total oxidant status; OSI, oxidative stress index; B, baseline; AT, after treatment

Table 2. Correlation coefficient values of the parameters

	TAC_b	TOS_b	OSI_b	TAC_at	TOS_at	OSI_at	Scale_b	Scale_at
TAC_b	1.000	−.211	.793**	.515**	−.136	.447**	.081	.112
TOS_b	−.211	1.000	−.702**	.056	−.124	.049	−.005	.034
OSI_b	.793**	−.702**	1.000	.280	−.046	.250	−.035	−.021
TAC_at	.515**	.056	.280	1.000	−.133	.766**	.086	.063
TOS_at	−.136	−.124	−.046	−.133	1.000	−.688**	−.101	−.276
OSI_at	.447**	.049	.250	.766**	−.688**	1.000	.130	.264
Scale_b	.081	−.005	−.035	.086	−.101	.130	1.000	.584**
Scale_at	.112	.034	−.021	.063	−.276	.264	.584**	1.000

** p<0.001 (strong correlation); * p<0.05 (correlation)
TAC, total antioxidant capacity; TOS, total oxidant status; OSI, oxidative stress index
b, baseline; at, after treatment

RESULTS

No statistically significant difference in baseline anxiety scale values was found between G1 and G2 (p>0.05). Anxiety values were decreased after treatment in both groups. While the anxiety scores obtained after dental procedure in G2 showed a statistically significant decrease compared with the baseline values (p<0.05), the decrease in the anxiety scoresin G1 was not statistically significant (p>0.05) (Table 1). In the analysis of baseline OS biomarkers, TAC and OSI values were higher in G1 than in G2, and this increase was statistically significant (p<0.05). After the current treatment, a significant decrease in TAC and OSI values was obtained in G1 (p<0.05). No significant difference in the values of OS biomarkers at baseline and after treatment was noted in G2 (p>0.05) (Table 1).

In the correlation analysis, a strong correlation was found between baseline TAC, TOS, and OSI values and post-treatment TAC and OSI values (p<0.001). Although no correlation exist between OS biomarkers and anxiety scale values (p>0.05), a strong correlation was found in baseline and post-treatment anxiety scale data (p<0.001) (Table 2).

DISCUSSION

Studies have shown that OS biomarkers increase in body fluids such as serum and saliva during or after stress. A study has also suggested that OS biomarkers are potential markers of stress (18). OS is considered an important component of various diseases. From the oxidation of deoxyribonucleic acid to proteins, lipids, and free amino acids, numerous methods have been developed and used in nearly all diseases to measure the extent and nature of OS (19). While OS measurements in the spinal cord and tissues are limited to certain diseases, measurements in venous blood and urine samples are the most common methods in clinical practice (20). As researchers show that saliva contains oxidation biomarkers similar to serum, saliva is increasingly used to measure OS markers (21). In addition, saliva analysis has been proposed as a noninvasive and low-cost method for screening OS (22). In pediatric patients, this method can be preferred to prevent potential stress that may occur during sample collection. Moreover, saliva is considered the first line of defense against OS (23). Therefore, in this study, saliva samples were used to evaluate the relationship between OS biomarkers and anxiety caused by dental treatment in children.

Many studies have shown the relationship between OS and periodontal diseases (23, 24), malignant oral disorders (25),various systemic diseases (14, 26), and dental caries (27, 28). However, to the best of our knowledge, no study has evaluated the relationship between dental experience-based anxiety and OS biomarkers. For this reason, this pilot study was conducted. In this study, children without dental treatment experience will have lower OS biomarker values than those with dental treatment

experience, as shown in the statistically significant increase in baseline TAC and OSI values in G1. TAC describes the combined ability of a group of enzymes, including saliva antioxidants, saliva peroxides, saliva uric acid, and several small enzymes (29). Saliva peroxidase catalyzes the peroxidation of thiocyanate ion to produce oxidation products. This prevents the growth and metabolism of many microorganisms, thereby inhibiting caries or at least slowing the progression of caries (27). In this study, increased TAC and OSI values in G1 may be due to the patient's past dental treatment experience being a surgical procedure. Statistically significant decrease in post-treatment TAC and OSI values in G1 also shows that dental anxiety affects OS biomarkers. Increased OS biomarker levels in a child who had undergone surgery decreased with more easily tolerated processes, such as preventive or restorative applications. In G2, no significant difference was found in baseline and post-treatment values of OS biomarkers. In this case, the hypothesis that there would be a significant decrease in OS biomarkers in both groups after the procedure could be rejected. However, a statistically significant decrease in anxiety scale value was found. Perhaps, saliva samples collected immediately after the procedure does not reflect any changes in OS biomarker levels. Similar to the present study, Zarbanand et al. (30) reported no significant change in TAC values before and after treatment. However, Al Anaziand et al. (28) reported a decrease in TAC values in saliva measurements at 1 week and 3 months after the treatment.

The third hypothesis that initial and post-procedure anxiety levels would correlate with OS biomarkers can also be rejected because no correlation was noted between these two parameters. However, considering other results of this study, this correlation could be achieved by increasing the number of patients. More comparable results could be obtained by increasing the number of patients, differentiating the dental treatment experience, and repeating saliva measurements at regular intervals after treatment.

This study has several limitations. First, this was conducted as a pilot study. Second, although dental anxiety scores were recorded, general anxiety scores and psychiatric stress were not evaluated. Third, even if the analysis was performed in a pediatric population, variation between age groups may affect the secretion of saliva. This could result in differences in salivary biomarkers.

CONCLUSION

Within the limitations of this study, increased OS biomarker values in patients who has undergone surgery decreased significantly after treatment. In patients without dental treatment experience, baseline and post-treatment anxiety scale data decreased, but no significant change in salivary parameters was observed. Although this finding is unclear, there may be a relationship between anxiety and OS biomarkers. Additional studies are needed to understand the relationship between dental anxiety and pathophysiological changes in OS biomarkers due to dental treatment experience.

Ethics Committee Approval: Ethics committee approval was received for this study from the Clinical Research Ethics Committee of Gaziantep University (2019/153).

Informed Consent: All participants were informed and consent forms were obtained.

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