Effect of Local Anaesthesia Immediately before Dental Treatment on the Relationship between Dental Anxiety and Salivary Cortisol Levels in Patients with ICDAS-II Score 5 Lesions

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Abstract

Background: Dental anxiety is a psychological and physical state of fear in patients experiencing stress over dental procedures. **Aim**: This cross-sectional study aimed to investigate the effects of local anaesthesia administered immediately before dental treatment on the association between dental anxiety and salivary cortisol levels in patients with International Caries Detection and Assessment System (ICDAS) score 5 lesions. **Materials and Methods**: A total of 95 patients aged between 16 and 45 years who had a caries lesion with an ICDAS-II score of at least 5 were examined at Istanbul University, Faculty of Dentistry, Department of Restorative Dentistry. At the beginning of operative procedures and just before the administration of anaesthesia, the Modified Dental Anxiety Scale was used to measure patients' dental anxiety, and enzyme-linked immunosorbent assay kits were used to assess patients' unstimulated salivary cortisol levels. The data were statistically analysed using the Mann–Whitney *U* test and Spearman correlation analysis $P < 0.05$. **Results**: The mean values between male and female patients for dental anxiety scores were statistically significant, 9.07 ± 3.07 and 12.42 ± 4.33, respectively ($P < 0.05$). However, salivary cortisol levels did not exhibit statistical significance; the mean values were 4.49 ± 2.31 and 3.95 ± 2.39, respectively ($P > 0.05$). There were significant correlations between age and salivary cortisol levels in female patients ($P < 0.05$), but there were no significant correlations for any of the parameters in male patients ($P > 0.05$). **Conclusions**: Within the limits of this study, patient gender had an influence on the extent of dental anxiety, and a significant correlation was found between age and salivary cortisol levels in female patients.

Keywords: Dental anaesthesia, dental anxiety, ICDAS, Modified Dental Anxiety Scale, salivary cortisol level

Introduction

Dental anxiety is a psychological and physical state of fear in patients who experience stress before dental procedures, which may partially or completely prevent a person from receiving dental treatment.[1,2] According to Thomson *et al.*,[3] exogenous factors are the main cause of dental anxiety, although endogenous factors can also contribute to this condition. Exogenous factors arise from patients' previous negative experiences with dental treatment, from hearing about others' negative experiences or from other information channels.[4] In addition, sensory triggers such as the appearance of needles and instruments, the characteristic smell of a dental clinic and the sounds and vibrations of instruments can also trigger anxiety.[5] Studies on the

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prevalence of dental anxiety in pediatric and adult patients demonstrate that it varies between 5.7% and 20.2% worldwide, while the prevalence in the Turkish population is between 21.3% and 23.5%.[6-8] According to studies conducted in Turkey and other countries, dental anxiety is significantly higher in women than in men.[4,6] Dental anxiety causes severe pain in patients, and the dentist may also suffer during the treatment of such patients.[8] The main cause of delaying dental treatment or missing a dental appointment is dental anxiety, which is a major obstacle to maintaining oral health. Missed dental treatment, in turn, can lead to tooth decay and social stigmatisation and in severe cases can even cause social isolation.[1]

Dental anxiety is generally measured using questionnaires and scales. The Dental Anxiety Scale is widely used in epidemiology and clinical research.[9] The Modified Dental Anxiety Scale (MDAS) was developed to improve the DAS and overcome its limitations. In particular, a question on injections was included in the MDAS. The MDAS uses five questions to assess patients' anxiety about dental treatment.[10] The response to each question is scored from 1 to 5 (1 = not anxious and 5 = extremely anxious).[10,11] A Likert scale is used in the MDAS, which gives a minimum score of 5 and a maximum score of 25.[10]

Cortisol is a glucocorticoid steroid hormone derived from cholesterol[12] and is a biomarker used to assess stress-related disorders. It is produced in the zona fascicula of the adrenal cortex.[13] Dental anxiety causes activation of the autonomic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis.[14] This activation causes the secretion of cortisol from the adrenal cortex into all body fluids, such as urine, serum and saliva.[15] Therefore, salivary cortisol levels may increase during stress and anxiety. Measuring salivary cortisol levels is a simple, reliable, accurate and noninvasive way to determine increased stress levels.[16] In dentistry, salivary cortisol levels can be measured to determine the level of dental anxiety.[17]

The aim of this study was to investigate the association between dental anxiety and salivary cortisol levels in a group of patients prior to operative dental treatment under local anaesthesia due to the presence of carious lesion with an ICDAS-II score of 5.

The null hypotheses tested were as follows: (1) no significant difference between male and female patients in terms of dental anxiety, (2) no significant correlation between salivary cortisol levels and dental anxiety and (3) no significant correlation between age and salivary cortisol levels.

Materials and Methods

Study protocol

The study was conducted among dental patients who regularly attended the clinics of Istanbul University, Faculty of Dentistry, Department of Restorative Dentistry for treatment. A total of 95 patients with an average age of 30 years (range = 16–45 years) who had at least one carious lesion with an ICDAS-II score of 5, verified by two independent examiners, were selected to participate in this cross-sectional study conducted at a single centre. The study complied with Good Clinical Practice guidelines, and the research protocol was ethically approved by the Istanbul University Faculty of Medicine (No. 2015/54). All patients were informed verbally and in writing about the study, and signed informed consent was obtained.

Sample size determination and patient selection

The sample size was determined by a power analysis at a power of 80% with a confidence level of 95% and a significance level of $P < 0.05$ (NCSS PASS 2000, Kaysville, UT, USA). The power analysis was performed based on a previous study in which the sample size was estimated.[17] The results of the power analysis indicated that at least 88 patients were required for the study, but to increase the power and account for the possible loss of patients, 111 patients were included in the study. Due to insufficient saliva number of samples ($n = 10$ patients) and the detection of contaminated blood in saliva ($n = 6$ patients), a total of 16 patients were not included in the study [Figure 1]. Of the 95 patients, 53 were female and 42 male. The study was conducted from January 2015 to May 2015.

Patients were included if they (1) were in good general health, (2) had no systemic health problems and (3) had at least one decayed tooth that had an ICDAS-II score of 5. Patients were excluded from the study if they (1) had a systemic disease that altered salivary flow, (2) were taking medications that caused hyposalivation, (3) were pregnant or breastfeeding, (4) had a known allergy to a local anaesthetic, (5) were addicted to alcohol and/or drugs or (6) were smokers.

Clinical procedure

The same experienced, specially trained investigator carried out all the data collection, data recording and saliva sampling procedures. To reduce the effects of the circadian rhythm on cortisol secretion, saliva samples were collected between 10:00 and 11:00 am. Before saliva sample collection, each patient was asked not to eat or drink anything at least 1 h before the session. Prior to saliva sample collection, each patient waited in a separate room for 15 min before being asked to enter the treatment room. Each patient was informed about the dental injection while sitting in the dental chair. Immediately after this information, saliva samples were taken, and the MDAS questionnaire was completed. Saliva was taken from the patients once shortly before administering anaesthesia, and the cortisol level was measured accordingly. To measure patients' anxiety about

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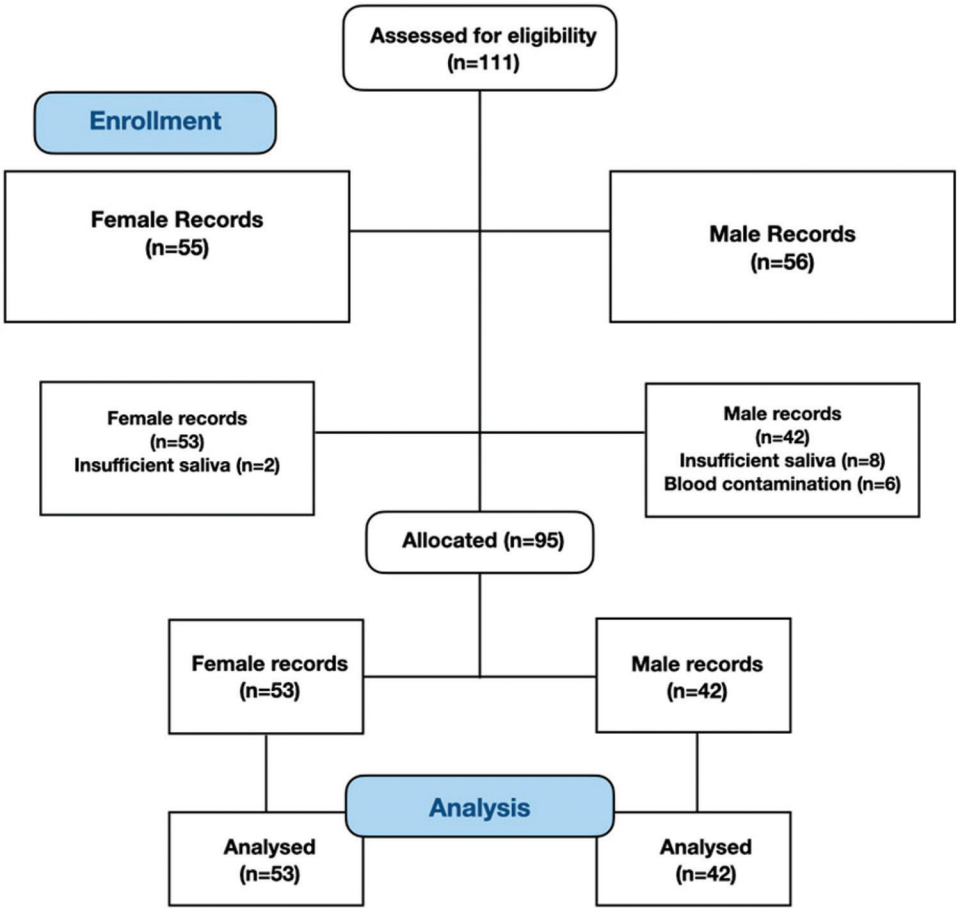

Figure 1: Flow chart of the study

dental injections, they were asked to rate their anxiety level on a 5-point scale in an MDAS questionnaire. The answers ranged from not anxious (score 1) to extremely anxious (score 5).

An unstimulated saliva sample was taken to determine salivary cortisol levels. Each patient was asked to place an absorbent cotton roll in the mouth for at least 3 min until it was soaked with saliva. All collected cotton rolls were carefully examined for possible blood contamination, and samples that showed a clear consistency were immediately transferred to a 5-mL plastic tube. The samples were stored at 4°C and centrifuged at 3000 rpm for 2 min on the same day. The saliva samples were aliquoted and stored at −20°C until the day of measurement. After thawing, the samples were centrifuged at 3000 rpm for 10 min. The test was performed according to the instructions of the salivary cortisol enzyme-linked immunosorbent assay (ELISA) laboratory kit (DRG International Inc., Springfield, NJ, USA; catalog number SLV-2930). Samples were measured at 450 nm using an absorbance microplate reader (ELx800, BioTek Instruments, Inc., Winooski, VT, USA). The results were automatically calculated using a logistic curve fit with four parameters.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software, version 21.0 (SPSS Inc., Chicago, IL, USA). The results were primarily analysed using the Kolmogorov–Smirnov test for normal distribution of data. Since the data were non-normally distributed, a Mann–Whitney *U* test was used to compare the MDAS and salivary cortisol levels of men and women. Spearman's rank correlation test analysed the correlations between age, MDAS and salivary cortisol levels within the female and male groups at $P < 0.05$.

Results

A total of 111 patients were initially included in this study. Due to insufficient saliva samples ($n = 2$ female, $n = 8$ male) and the detection of contaminated blood in saliva ($n = 6$ male), 16 patients were excluded from the study. Figure 1 shows the flow chart of the study. Of the 95 patients who met the inclusion criteria and participated in the study, 53 were female, with a mean age of 30.68 ± 8.06 years (range = 16–45 years), and 42 patients were male, with a mean age of 29.62 ± 7.32 years (range = 16–43 years). Table 1 summarises the demographic data of the included patients. Table 2 summarises the comparisons

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of MDAS and salivary cortisol values between male and female patients. The mean MDAS values for the male and female patients showed statistically significant differences, recorded as 9.07 ± 3.07 and 12.42 ± 4.33, respectively ($P < 0.001$). The mean salivary cortisol levels (ng/mL) for male and female patients were 4.49 ± 2.31 and 3.95 ± 2.39, respectively, and there were no significant differences in the salivary cortisol levels ($P > 0.05$). Table 3 summarises Spearman's rho correlations of MDAS, age and salivary cortisol levels between male and female patients. There were significant correlations between age and salivary cortisol levels for the female patients ($r = -0.432$, $P < 0.001$), but there were no significant differences between age and MDAS scores ($r = 0.145$, $P > 0.05$). There were no significant correlations in any of the parameters of the male patients.

Discussion

Dental anxiety affects most people of all ages and social classes. They completely avoid dental treatment, dental check-ups or cooperation with dentists, resulting in poor oral and dental health.[18] It may be caused by the reactions of the family and social environment, general fear, pain or traumatic experiences.[19] The most common demographic factors associated with differences in anxiety levels are gender and age.[2,4] Caries-related chronic pain in patients and dental procedures may lead to increased levels of chronic stress.[20] In this study, we aimed to investigate the effect of local anaesthesia administered immediately prior to dental treatment on the correlation between dental anxiety and salivary cortisol levels in patients with distinct cavities with visible dentin walls (ICDAS score of 5).

It was found that the mean MDAS score was significantly higher in female patients. According to MDAS, values <11 are considered normal, 11–18 are moderately anxious and those >19 are considered excessively anxious.[21] According to these values reported in our study, normal dental anxiety levels were observed in male patients and moderate dental anxiety levels in female patients. Although we observed that some patients have high MDAS scores, we believe that not seeing high anxiety levels in terms of mean values is why patients routinely undergo dental faculty clinics for dental treatment. These findings are in line with those of the subsequent studies in which gender significantly influences dental anxiety.[11,22] The reason for this may be that females explain their psychological states more comfortably than males and that they are more sensitive about this, causing this difference between genders. This sensitivity was investigated, and the volume of grey matter activated during emotional regulation was higher in females.[23] However, Kanegane *et al.*[17] stated that there is no association between gender and dental anxiety, and they linked this difference to the small sample size of their study.

Saliva cortisol level measurement is a reliable indicator in evaluating HPA activity[24] because HPA activity induces the release of adrenocortical hormones during

Table 1: Demographics of the included patients

Gender	*n*	Age (Mean ± SD)	Minimum	Maximum
Female	53	30.68 ± 8.06	16	45
Male	42	29.62 ± 7.31	16	43

SD: standard deviation

Table 2: Mean dental anxiety (MDAS) and salivary cortisol levels according to gender

	Gender	Mean ± SD	Minimum	Maximum	*P*
MDAS	Female	12.42 ± 4.33	5.00	22.00	**0.001**
	Male	9.07 ± 3.47	5.00	17.00	
Cortisol	Female	3.95 ± 2.39	1.41	12.35	0.065
	Male	4.49 ± 2.31	2.03	12.17	

Mann–Whitney *U* test ($P < 0.05$). SD: standard deviation, MDAS: Modified Dental Anxiety Scale

Bold value indicates $p < 0.001$

Table 3: Spearman's rho correlation analysis result (cross-comparison)

Gender		Age	MDAS	Cortisol
Female	Age		*r* 0.145	*r* −0.432
			P = 0.301	***P* < 0.001**
	MDAS	*r* 0.145		*r* 0.005
		P = 0.301		*P* = 0.971
	Cortisol	*r* −0.432	*r* 0.005	
		***P* < 0.001**	*P* = 0.971	
Male	Age		*r* −0.113	*r* −0.008
			P = 0.476	*P* = 0.962
	MDAS	*r* −0.113		*r* 0.173
		P = 0.476		*P* = 0.274
	Cortisol	*r* −0.008	*r* 0.173	
		P = 0.962	*P* = 0.274	

MDAS: Modified Dental Anxiety Scale

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emotional stress situations such as dental anxiety.[25] The advantage of using saliva samples is the noninvasive ease of collection and even low concentrations in saliva can be determined using various sensitive methods.[26] The ELISA immunoassay method was used for saliva sample analysis and interpretation in our study.

It has been reported that mechanical trauma of needle insertion during intra-oral local anaesthesia or sudden injection of the anaesthetic solution into the soft tissue is the most anxiety-provoking procedure for patients.[27] Moreover, duration and type of treatment and general or local anaesthesia applications have been reported to affect cortisol concentration in saliva.[28] Also, previous experiences of patients about anaesthesia applications or hearing from others about these applications provoke anxiety and thus increase cortisol concentrations. In our study, saliva was collected after filling out the MDAS form and before the application of local anaesthesia. According to the findings of our study, no significant difference was found between the mean saliva cortisol concentrations based on gender. This result is similar to those of some studies.[17,29] In addition, there was no significant correlation between MDAS and saliva cortisol levels in both sex groups. Similar to our study, Sadi *et al.*[30] did not find statistically significant correlations between DAS and saliva cortisol levels. Although there was a significant difference between the mean MDAS scores by gender, the difference between saliva cortisol levels may be due to the self-restrictions of males to express their anxiety.

In the present study, for patients with the mean age was 30 years and the age range of 16–45 years, no statistically significant correlation was found between age and MDAS scores. However, a high level of significant correlation was found between age and saliva cortisol concentrations in female patients. Although there is no correlation between age and MDAS, high cortisol levels observed due to dental anxiety in young females may have influenced this result. However, no statistical analysis was performed in this study based on age groups. Similar to the findings of our study, Yakar *et al.*[22] did not find a statistically significant difference in the MDAS scores between individuals aged under 40 and above in their study.

This study had some limitations. Because the MDAS is a scale with few questions, it is prone to error. While there are more advanced scales for assessing dental anxiety, they require much more time to complete. In addition, the data obtained with the MDAS can be sorted ordinally. However, the distance between these classifications is not linear. The cross-sectional design of the study is another limitation that does not allow causality to be established. In addition, only one saliva sample was taken from the patients and only a few demographic variables (age and gender) were measured.

Further studies are needed to determine the correlation between dental anxiety levels and salivary cortisol concentration, using various demographic factors, a wider age range and more patients.

Conclusions

Within the limitations of the present study, the first null hypothesis was rejected that there would be no difference between MDAS scores based on gender. Higher levels of dental anxiety have been observed in females. The second null hypothesis tested was accepted to have no correlation between saliva cortisol levels and dental anxiety. The third null hypothesis tested is partially accepted that there will be no correlation between age and saliva cortisol levels. While higher saliva cortisol concentrations were observed in female patients with decrease in age, there was no significant correlation in male patients.

Cortisol level in saliva with MDAS, which allows assessment of local anaesthetic fear, is a useful marker in determining dental anxiety and may be preferable for determining dental anxiety levels according to various demographic factors.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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