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Salivary Cortisol Measurement as a Test for Dental Anxiety Before Tooth Extraction

Ocena stężenia kortyzolu w ślinie dla oceny stresu pacjenta przed zabiegiem ekstrakcji zęba

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Dental procedures are a source of anxiety, which is why most patients consider dental treatment unpleasant. Cortisol is a steroid hormone with wide effects on metabolism. Increased cortisol concentration is observed in stressful situations.
Objectives. The aim of the study was to evaluate the level of anxiety before tooth extraction, both in patients' subjective opinion as well as in cortisol concentration in patients' saliva before the procedure.
Material and Methods. Saliva for the examination of cortisol concentration was gathered from 44 patients (21 males and 23 females) before tooth extraction. The control group consisted of 31 persons between the ages of 18 and 64 (11 males and 20 females). The Corah's Dental Anxiety Scale (CDAS) was used to estimate dental fear. Cortisol concentration was determined by means of electrochemiluminescence (ECLIA).
Results. The average cortisol concentration in patients' saliva before tooth extraction was 0.50 ± 0.23 μg/dL which is within the 0.04 μg/dL – 1.23 μg/dL range. The average cortisol level in saliva in the control group was 0.18 ± 0.10 μg/dL. The difference in cortisol concentration between both groups was statistically significant. Statistical analysis of cortisol concentration in different age groups showed no significant correlation between the concentration of cortisol and age. No statistically significant difference in cortisol concentration before tooth extraction in women and men was noticed. The concentration of cortisol in men had more often extreme values, which is different from the results obtained in women. The highest concentration of salivary cortisol was found in the group declaring low level of anxiety by CDAS. However, there was no statistically significant correlation between salivary cortisol and level of anxiety measured by CDAS.
Conclusions. Increased salivary cortisol concentration showed patients' anxiety before tooth extraction. The level of anxiety declared by patients before tooth extraction did not show any significant correlation with salivary cortisol concentration **(Dent. Med. Probl. 2015, 52, 4, 408–414).**

Key words: cortisol, saliva, dental anxiety.

Słowa kluczowe: kortyzol, ślina, lęk dentystyczny.

Dental procedures are a source of anxiety for many people. Most patients consider dental appointments unpleasant [1]. Dental anxiety is considered to be a natural reaction of the body, and its origin is multifactorial. Fear of pain which the patient may experience in the dental office is a major cause of anxiety. Other factors that may cause anxiety are noise and vibration of dental drills, the appearance of dental instruments, bad taste and odor of drugs, immobilization in dental chair, and lack of information about the treatment. Anxiety or fear associated with dental procedures is a source of patient's oral problems and it makes it difficult for the physician to perform surgery [2, 3].

Dental anxiety is a reaction to the unknown threat. It is very common and most people experience it in varying degrees before surgery, especially if they had not been treated in this particular way before. Dental fear appears in response to a known threat and leads to avoiding or escaping from the dental appointment, which is also known as dental phobia. A person with a phobia avoids dental treatment until the pain and panic overtake or until there is a change in the psychological barrier [4]. Dental phobia is a paralyzing fear of the dentist. Dental phobia may result from negative experiences suffered in childhood, fear of pain or a feeling of helplessness and lack of control during dental procedures [5].

Regardless of the reasons and factors causing anxiety it is considered at three levels: emotional, vegetative and behavioral. Emotional anxiety is a sense of danger and uncertainty together with exasperation, annoyance, waiting for the unpleasant event, feeling light-headed, impaired concentration and fear of loss of self-control [3, 6].

Somatic vegetative symptoms are associated with increased activation of the sympathetic nervous system, and thus tachycardia, dry mucous membranes, increased blood pressure, shallow breathing, discomfort in the area of the heart and/or stomach, excessive sweating, heat stroke, pale skin, increased bowel motion, intestinal spasms, diarrhea, nausea, paresthesia of the limbs, dizziness and metabolic changes: hyperglycemia and increased cortisol secretion appear [3, 7].

Behavioral anxiety causes changes in facial expressions (anxiety, pain, fear, horror) and restlessness (manipulative, locomotor) and immobility or verbal phenomena – scream, moan [3].

Cortisol (hydrocortisone) is a natural steroid hormone and the main representative of glucocorticoids. It is composed of 11-β-deoxycortysol by 11-β-hydroxylase activity in the adrenal *zona fasciculata*. Cortisol causes an increase in blood glucose level by releasing amino acids from peripheral tissues and accelerating glucogenesis, as well as the inhibition of skeletal glucose metabolism and acceleration of the breakdown of fatty acids to ketones. Cortisol also has anti-inflammatory effects. There is a synthetic equivalent thereof, i.e. cortisone, which is converted in the liver into cortisol [8, 9].

Under physiological conditions, adrenal glands produce a daily about 10–30 mg of cortisol. The highest level in the blood occurs in the morning. In stressful conditions, this level may be increased [10, 11].

In order to determine the cortisol level usually blood is taken. Blood samples should be taken at 8 am, when cortisol concentration is at its peak and again at about 4 pm, when the level should be low.

Concentration of cortisol can also be tested in a sample of saliva. Although saliva sampling is less stressful for the patient than blood collection, however, the procedure requires special laboratory methods and, therefore, the study of cortisol in saliva is not widely used [12]. However, the development of diagnostic methods and new analytical techniques make saliva a popular subject to laboratory tests. In many centers salivary tests are developed instead of blood serum to determine the concentrations of different compounds and biochemical substances.

Obtaining blood test is an invasive method, requiring trained medical personnel, and also creates the possibility of accidental transmission of viral diseases (HCV, HIV). The simplicity of saliva collection, where any quantity of material can be obtained, is non-invasive and repeatable, making the diagnostic methods carried out in human saliva attractive. Salivary tests are reliable laboratory tests substituting biochemical determination of selected compounds in the blood serum or urine [12–15].

A saliva sample is usually taken by spitting and drooling. Sometimes, in order to stimulate secretion of saliva, it is recommended to chew pellets of wax or paraffin [16].

The use of a saliva collection kit *Salivette*® is the best option for aesthetic and hygiene reasons. A patient after rinsing his mouth with water, puts *Salivette* cotton roller in the mouth, which absorbs the saliva. Jaw movements stimulate the secretion of saliva. Saturated saliva roller is inserted back into the *Salivette* tube, sealed, labeled and sent to the laboratory.

Material and Methods

The group considered for the study were patients qualified for tooth extraction in Oral Surgery Department of Medical University of Lublin, Poland.

The examination group (Group A) consisted of 44 patients aged between 18 and 64 (21 males and 23 females), who had saliva taken before tooth extraction. The patients had no hormonal disorders, did not take any hormonal medication and reported no gingival bleeding.

The control group (Group C) consisted of 31 people aged between 18–64 years of age (11 men and 20 women), who had no dental treatment on that day and from which saliva was collected for determination of cortisol concentration. These patients, as in Group A, did not report systemic diseases, medication, hormonal disorders and gingival bleeding.

Saliva samples were collected in the morning between 8.00 am and 10.00 am. *Salivette* sets were used. The patients was first told to rinse their mouth with water, then a cotton swab was placed under the tongue and the patient was told to perform chewing movements. After 3–5 minutes, the swab soaked in saliva was placed in a test tube, which was sealed and numbered. The tubes of the test material was stored at –20°C. Then the saliva was thawed and centrifuged for 20 minutes at 1000 g overload (about 2400 revolutions/minute). The supernatant was the material to the determination of cortisol concentration.

For the determination of cortisol in saliva Roche Elecsys 2010 unit was used. Cortisol concentration in saliva was determined by means of electrochemiluminescence (ECLIA) [25].

The Corah's Dental Anxiety Scale (CDAS) was used to estimate dental fear. CDAS questionnaire, a brief four-item scale, is considered to be a reliable, valid, and useful measure of dental anxiety.

The results of the survey were analyzed statistically. Descriptive statistics for the value of the measured parameters is shown by the mean, standard deviation, median (for continuous variables), and the number and percentage (for nominal variables). In cases of improper assumptions required for the use of parametric tests, nonparametric tests were used. To compare two independent groups Mann-Whitney U test was used. To investigate the covariance between variables Spearman rank correlation was used.

Acceptable probability of error of the first kind (the intensity level of the test) was assumed at $p = 0.05$.

Results

The mean concentration of cortisol in the study was 0.50 ± 0.23 μg/dL. This value ranged from 0.04 μg/dL to 1.23 μg/dL. It was noticed that the distribution of average concentrations of salivary cortisol of patients in study group (Group A) was a normal distribution (Tab. 1, Fig. 1).

Distribution of the level of cortisol in the saliva of individuals in the control group (Group C) also did not differ significantly from the normal distribution (Fig. 2).

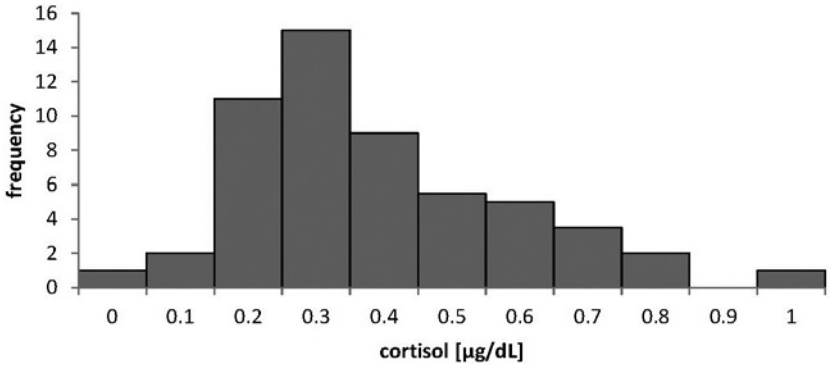

Fig. 1. Distribution of cortisol concentration (μg/dL) in patients in the study group (Group A)

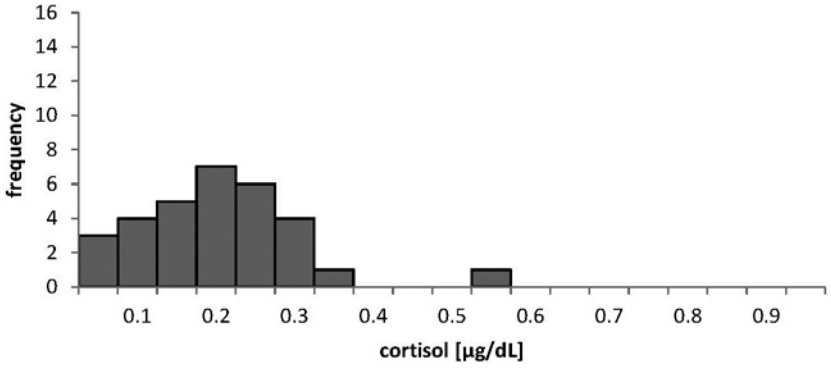

Fig. 2. Distribution of cortisol concentration (μg/dL) in patients in the control group (Group C)

Table 1. Distribution of cortisol concentration (μg/dL) in patients of both groups

Group	N	Mean μg/dL	Std. deviation	Minimum	Median	Maximum
Study group (A)	44	0.50	0.23	0.04	0.45	1.23
Control group (C)	31	0.18	0.10	0.18	0.18	0.52

Average and median levels of cortisol in the saliva of Group C were 0.18 μg/dL. Maximum values were 0.52 μg/dL (Tab. 1).

Group A was divided into subgroups: subgroup A0 under 25 yr of age (n = 11), A1 between 26 and 40 yr of age (n = 23), A2 over 40 yr of age (n = 10).

Variations in salivary cortisol concentration between patients of Group A and the Group C occurred in all age groups. In each age group, cortisol levels were higher in patients before tooth extraction (A0 – under 25 yr of age – 0.51 μg/dL, A1 – 0.50 μg/dL, A2 – 0.52 μg/dL), and for the control group it was 0.17 μg/dL, 0.18 μg/dL, 0.19 μg/dL). Distribution of average cortisol concentrations in different age groups in patients in both study group (A) and control group (C) is shown in Fig. 3.

The average value of the concentration of cortisol in Group A in both genders was 0.50 ± 0.23 μg/dL. The average for men was 0.51 ± 0.25 μg/dL, and for women it was 0.50 ± 0.21 μg/dL. Statistical analysis showed no statistically significant differences in salivary cortisol levels before tooth extraction between men and women (p = 0.76) (Tab. 2).

In the control group (Group C) the average concentrations of cortisol were similar, slightly higher in women (0.18 ± 0.12 μg/dL) than in men (0.18 ± 0.07 μg/dL), however the difference was not statistically significant (p = 0.79) (Tab. 2).

Significant variations in the concentration of salivary cortisol occurred between women in group A and group C and between men from both groups (p < 0.001). The level of cortisol was higher in males (0.51 μg/dL) than for females (0.50 μg/dL). In the Control group, cortisol levels were 0.18 μg/dL for males and females (Tab. 2, Fig. 4).

The highest level of cortisol in saliva in Group A was observed in patients who reported no fear of tooth extraction according to the CDAS scale (average concentration 0.57 ± 0.39 μg/dL). Lower values (0.49 ± 0.19 μg/dL) were observed in patients with low anxiety according to the CDAS scale. The concentration of salivary cortisol in patients classified according to the scale CDAS to a group with medium and high level of anxiety were 0.48 μg/dL.

In the control Group (Group C) the highest levels of cortisol (0.20 ± 0.12 μg/dL) were recorded in patients with low levels of anxiety claimed by the CDAS scale and the lowest value (0.16 ± 0.09 μg/dL) was recorded in the group of people with an average level of anxiety according to CDAS.

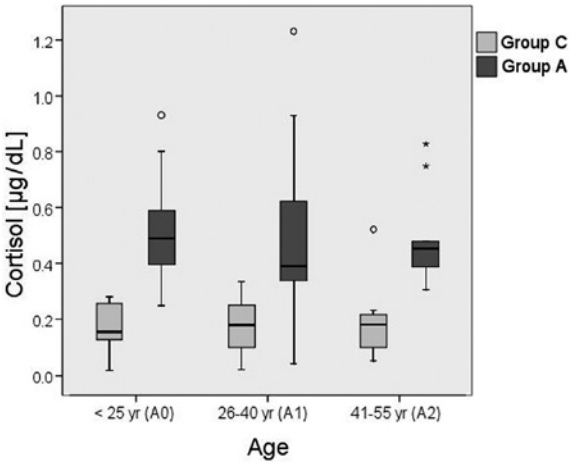

Fig. 3. Distribution of average cortisol values in different age groups in patients in study group (Group A) and control group (Group C)

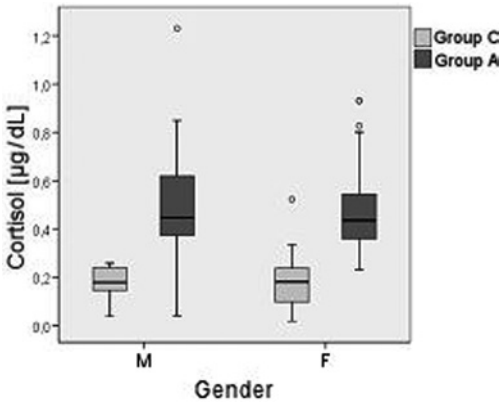

Fig. 4. Cortisol level in men and women in Group A and Group C

Table 2. Mean cortisol concentration in men and women

Group	Gender	N	Mean μg/dL	Std dev.	Min	Median	Max	Stat. analysis*
Group A	M	21	0.51	0.25	0.04	0.45	1.23	U = 228 z = 0.32 p = 0.76
	F	23	0.50	0.21	0.23	0.44	0.93	
	general	44	0.50	0.23	0.04	0.45	1.23	
Group C	M	11	0.18	0.07	0.04	0.18	0.26	U = 103 z = –0.29 p = 0.79
	F	20	0.18	0.12	0.02	0.18	0.52	
	general	31	0.18	0.10	0.02	0.18	0.52	

* Mann-Whitney U-test.

Table 3. Comparison of cortisol levels between the group of patients (group A) and control group (Group C) with the level of dental anxiety by CDAS

Level of anxiety (CDAS)	Group/ cortisol	n	Mean rank	Sum of ranks	U Mann--Whitney	W Wilcoxon	Z	p
No anxiety	C	11	7.00	77.00	11.00	77.00	–2.49	p = 0.011
	A	7	13.43	94.00				
Low anxiety	C	15	9.80	147.00	27.00	147.00	–4.48	p < 0.001
	A	25	26.92	673.00				
Medium anxiety	C	5	3.00	15.00	0.00	15.00	–3.12	p < 0.001
	A	11	11.00	121.00				

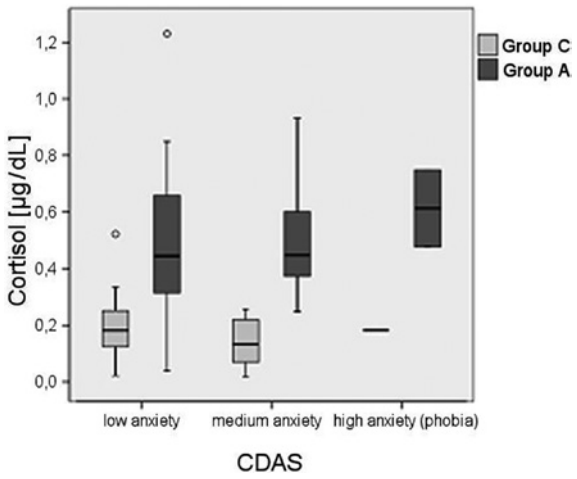

Fig. 5. The level of cortisol in saliva in subjects with different levels of anxiety in Group A and Group C

Significant variations in the concentration of salivary cortisol were noticed between Group C and Group A, which according to the scale CDAS were qualified for the group with lack of fear, anxiety and low average anxiety ($p < 0.013$, $p < 0.001$, $p < 0.002$). Average level of cortisol in saliva was higher in all patients prior to tooth extraction, regardless of the level of anxiety assessed by CDAS scale than in the control group (Tab. 3, Fig. 5).

Discussion

The level of salivary cortisol has been determined in various studies of dental anxiety. The cortisol level was determined before conservative treatment in adults and children, as well as in patients during periodontal treatment [8–20].

Brand [18] studied the relationship between the level of dental anxiety measured according to CDAS scale and level of pain intensity by VAS as well as the level of cortisol in saliva and urine of 76 patients before dental treatment. Studies have shown that the level of cortisol in saliva did not correlate with the level of anxiety according to CDAS scale and expected pain intensity according to VAS in such way as cortisol determined in the urine of patients. Elevated levels of cortisol remained longer in urine than in saliva even after the treatment. Therefore, the author claims that stress is reflected in the human metabolism.

Kanegane et al. [19] studied salivary cortisol levels in 76 adult patients and dental anxiety by CDAS and expected pain intensity by VAS before dental treatment. They received expected intensity of pain in men at 2.9 ± 3.4 points, and in women 4.18 ± 3.8 points. The level of cortisol in saliva in males (n = 34) was 2.02 ± 1.33 nM, while in women (n = 39) it was lower, 1.21 ± 1.30 nM. Cortisol level in patients reporting dental anxiety (n = 30) was 1.33 ± 1.30 nM, and in patients declaring no anxiety (n = 43) the level was higher up to 1.70 ± 1.35 nM. In patients expecting strong pain during the treatment concentration of cortisol was 2.14 ± 1.28 nM; however, in patients expecting low pain intensity cortisol concentration was 1.00 ± 1.30 nM. The authors found no relationship between expected pain intensity and concentration of cortisol in saliva. No relationship was found between the level of anxiety and concentration of cortisol in saliva as well. According to the authors increase in pain intensity during dental treatment contributes to an increase in salivary cortisol concentration.

Krueger et al. [2] studied the relationship between level of anxiety by CDAS and the concentration of cortisol in saliva of 19 patients. The evaluation was carried out in patients during clinical examination before periodontal treatment and during the treatment. In patients with high levels of anxiety by CDAS cortisol concentration was unchanged both before and during the therapy, but it was significantly higher than the concentration of cortisol in saliva in patients who declared low levels of anxiety.

Blomqvist et al. [21] conducted a study in 89 healthy thirteen-year olds and in 18 children aged 13 with ADHD (n = 18), determining the level of dental anxiety by CDAS and the level of cortisol in saliva during dental treatment. In both groups the

level of anxiety was elevated, and cortisol concentration in children with ADHD was significantly lower when compared to that of healthy children. The level of anxiety in children with ADHD measured by CDAS could be different not only due to the nature of the disease, but also because of lower reactivity to stress.

Greabu et al. [8] examined the concentration of cortisol in patients treated for periodontal disease (n = 36). The authors found that the concentration of cortisol in saliva was dependent on the type and duration of the surgery and anesthesia. They found that the treatment of periodontal disease cause stress and increased salivary cortisol concentration.

Albanidou-Farmaki et al. [17] conducted the study in 76 patients with recurrent aphtous stomatitis. They found that patients suffering from RAS had an elevated concentration of cortisol in saliva (average 1.44 ± 0.58 μg/dL) compared to the control group (0.91 ± 0.56 μg/dL), which suggests that stress can be an important factor in the pathogenesis of recurrent aphtous stomatitis.

In the literature there are few studies on the concentration of salivary cortisol before oral surgical procedures, such as tooth extraction. Miller et al. [22] studied 50 healthy men aged between 18 and 53, from which 196 samples of saliva were taken before dental procedures. Cortisol concentration ranged from 0.1 μg/dL to 3.8 μg/dL. The highest values of cortisol were reported before tooth extraction (1.09 ± 0.42 μg/dL) ($p < 0.05$). Significantly lower values were reported before dental examination (0.46 ± 0.10 μg/dL), scaling (0.64 ± 0.64 μg/dL), during conservative treatment (0.60 ± 0.04 μg/dL) and during endodontic treatment (0.49 ± 0.07 μg/dL). Analysis of the results shows that stress before tooth extraction is much stronger than associated with other dental procedures.

Hill and Walker [23] marked the level of cortisol in saliva in patients before surgical extraction of a wisdom tooth under general anesthesia (n = 27) and local anesthesia (n = 27). Samples of saliva were collected before and after the treatment as well as anxiety level was evaluated according HAD questionnaire (Hospital Anxiety and Depression). The authors showed that in patients who underwent surgery under local anesthesia salivary cortisol concentration was lower, however, level of anxiety by HAD scale was higher while compared with the results in patients undergoing surgery under general anesthesia. The authors concluded that the assessment of stress in patients on the basis of questionnaire survey is unreliable.

Similar studies assessing stress in patients prior to surgical wisdom tooth extraction under local and general anesthesia were conducted by Coulthard [24]. He took saliva samples from patients three days prior to surgery, on the day of treatment and three days after surgery and determined mean levels of concentration of salivary cortisol. Studies have shown that the concentration of salivary cortisol peaked on the day of treatment. However, the level of cortisol in saliva in patients who underwent the procedure under local anesthesia did not differ significantly in all tested samples. Patients in the study were allowed to determine the type of anesthesia they wanted. The author concluded that patients who chose surgery under general anesthesia showed significantly higher level of anxiety (according to HAD scale) on the day of surgery. In addition, he claimed that if patients were randomly qualified for surgery under general anesthesia, the level of cortisol in saliva would be similar. Therefore, according to the author there is no evidence that the procedure under general anesthesia is more stressful than the surgery under local anesthesia.

In our study ECLIA method was used to determine the concentration of salivary cortisol in patients before tooth extraction. Average cortisol concentration in the Group A (n = 44) was 0.58 ± 0.32 μg/dL, while the average concentration of cortisol in the control group (n = 31) was 0.18 ± 0.10 μg/dL. Statistical analysis showed significant differences between the group of patients (group A), and the control group (Group C) ($p < 0.0001$). Studies have shown that patients before tooth extraction had significantly higher concentration of cortisol than those in the control group. Similar results were obtained by Miller et al. [22], who found higher concentration of cortisol in saliva in patients prior to tooth extraction compared to the control group, and the highest level of cortisol before tooth extraction compared to other dental procedures.

In the study the distribution of cortisol in saliva, both in the group of patients before tooth extraction (Group A) and in the control group (Group C) had features of a normal distribution by Gaussian.

Statistical analysis of cortisol concentration in different age groups in patients from group A showed no significant correlation between concentration of cortisol and age.

No statistically significant difference in cortisol concentration before tooth extraction in women and men was noticed. The concentration of cortisol in men had more often extreme values, which is different from the results obtained in women.

Interestingly, in our study the highest concentration of salivary cortisol was found in the group declaring low level of anxiety by CDAS. However, there was no statistically significant correlation

between the level of cortisol in saliva and the level of anxiety measured by CDAS.

A significant increase in the concentration of cortisol in saliva in patients before tooth extraction indicates stress associated with this type of dental treatment. However, the increase in concentration of cortisol in saliva related to surgery and tooth extraction does not depend on the patient's age or gender.

The level of anxiety declared by CDAS before tooth extraction does not reflect patient experience of stress of surgical treatment.

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