



Influence of dental extraction on patient's stress and anxiety levels by assessing the salivary cortisol concentration at different time points during the extraction procedure

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Abstract

The aim of our study was to analyze the stress and anxiety in patients undergoing routine dental extraction, by the assessment of cortisol in saliva, which can be considered as one of the major hormones that is released during stressful events.

After obtaining the ethical approval and the patients were consented for their role in this study, we examined 26 patients (13 males, and 13 females). The age ranged from 29-42 with mean age of 36.2. We collected three salivary samples from each patient after asking him to chew on a cotton roll to stimulate salivary secretion. The first sample was obtained 10 minutes prior to local anesthetic injection, the second sample collected 6-8 minutes after the local anesthetic injection, and third sample acquired 10-15 minutes post-extraction. The samples were assessed and analyzed using ELISA immunoassay (competitive inhibition enzyme immunoassay technique). Statistical analysis was performed using SPSS statistical software (version 19). ANOVA and t-test were applied to obtain the significance and correlation between the examined samples.

Significant difference was shown among the three samples (P-Value =0.002).

The mean value in the third sample (post-extraction salivary sample) was considerably high when compared to the other samples (Mean \pm SD = 0.184 \pm 0.184, 0.307 \pm 0.121 and 0.357 \pm 0.141) respectively, which positively correlates with the patient's level of stress. Cortisol concentration in $\mu\text{g/dl}$ in percentages between each sample and the percentages, 50%, were 0.105 $\mu\text{g/dl}$, 0.295 $\mu\text{g/dl}$ and 0.410 $\mu\text{g/dl}$, (75 %) were 0.348 $\mu\text{g/dl}$, 0.410 $\mu\text{g/dl}$ and 0.443 $\mu\text{g/dl}$ and (90%), 0.443 $\mu\text{g/dl}$, 0.468 $\mu\text{g/dl}$ and 0.518 $\mu\text{g/dl}$.

Our study revealed that salivary cortisol is within its highest levels after extraction in 15 minutes.

Keywords: salivary cortisol, stress, anxiety, dental extraction, ELISA.

Introduction

Stress is complicated between the psychological and physiological effect on cortisol and adrenocortical axis. One of the hallmarks of the

hypothalamic-pituitary-adrenal (HPA) axis is its central role in the stress response. The adaptation to physical or psychological stress usually involves

the activation of the hypothalamus-pituitary-adrenal axis. The resulting release of hypothalamic corticotropin-releasing hormone, anterior pituitary adrenocorticotrophic hormone, and adrenal glucocorticoids (cortisol) in anticipation of stressful situations, interpreted as a homeostatic response of the body^(1, 2). Stress process can have a wide effect on body function; Stress experienced chronically can have widespread negative effects on a number of physiological systems, including reproduction⁽³⁾, growth⁽⁴⁾, and metabolism⁽⁵⁾, immune function⁽⁶⁾, and behavior⁽⁷⁾. In dentistry the acute effect of stressful events during the dental treatment can have a strong implication on our patients which reflects both the psychological and physiological components of stress; grave consequences can be related to anxious occasions during the course of the treatment which will influence the dentist and the patient's wellbeing. The increased metabolic demands throughout the stressful situations will have a dramatic impact on the patient general health especially on the medically compromised⁽⁸⁾.

Saliva had been studied extensively in the past decades to be used as non-invasive approach for measuring hormones and other body biomarkers. The assessment of cortisol in saliva has proven to be a valid and reliable reflection of the respective unbound hormone in blood. To date, assessment of cortisol in saliva is a widely accepted due to several advantages over blood cortisol analyses, the stress-free sampling, laboratory independence, and lower costs. The salivary cortisol assessment can be the method of choice in basic research and clinical environments^(9, 10).

Dental extraction procedure is one of the most dental treatments that the dentist will encounter during the daily clinical work. The procedure had been

considered as one of the main stress and anxiety inducers within the clinical dental settings^(11, 12).

Cortisol concentration in saliva when compared to serum (plasma) cortisol has shown to be less prominent⁽¹³⁾; however, the utilization of accurate laboratory technologies with high accuracy and the appropriate analysis with the proper data interpretation can enable researchers and clinicians to embrace the salivary cortisol as an approved method in the assessment of patient's stress and anxiety. (Radioimmunoassay)RIA and ELISA immunoassay both can be used as accurate methods in salivary cortisol assessment^(14, 15).

In this study, we intended to analyze the stress and anxiety in patients undergoing routine dental extraction, by the assessment of cortisol in saliva, which can be considered as one of the major hormones that is released during stressful events. We examined the cortisol levels in three different time points throughout the course of the extraction procedure to estimate at which stage the patient will be more prone to anxiety and stress.

Material and methods

This study was approved by the oral surgery department ethical committee, college of dentistry/Al Mustansiria University. We depended on informed consent form which had been acquired from all the patients prior to the conduction of the study process.

The study was conducted as collaboration between the department of oral surgery and the department of oral medicine and oral diagnosis; the tasks were distributed between the two departments. The case history form (Figure-1) was determined by the seniors' clinicians from both

departments. All the patients were first examined in the oral medicine and diagnosis department which then been referred to the oral surgery department to carry out the extraction procedure in accordance with previously approved case history form which encompassed the inclusion and exclusion criteria of the research.

The parameters that have been followed in our study were all in close relation to factors that may have an influential effect on the cortisol levels in the body. The inclusion and exclusion criteria were tailored in dependency with the major elements that may alter the cortisol levels within the body.

Twenty-six patients were incorporated in the study (13 male and 13 female patients) any female patient that was pregnant or taking contraceptive pills was excluded from the research. The age of the patients included was ranged between 29 to 42 years old with mean age of 36.2 years old. Social history such smoking and alcohol consumption were obtained as well, patient with chronic alcoholism were excluded from our research because alcohol can have an adverse effect on cortisol levels by affecting the HPA axis through release of endogenous components that may stimulate dopamine⁽¹⁶⁾. We excluded any unemployed patient from our research as that may have an influence on the body cortisol levels especially if the patient is suffering from long-term unemployment⁽¹⁷⁾. Other important social history factor that may affect the cortisol level is the marital status; we excluded any patient that is divorced or in the process of divorce announcement because we anticipated that this group of patients may be suffers from stressful life style which in turn may have an influential effect on cortisol levels⁽¹⁸⁾. Drugs history was evaluated and any patient that is/was

taking drug that may have influence on the HPA axis (such as steroids, psychotropic drugs, and/or patients chronically on non-steroidal anti-inflammatory drugs)^(19,20) were excluded. The chief complaint standardization was one of the major points in our inclusion and exclusion criteria we only included patients, who were suffering from pain, and we utilized the visual analogue scale (VAS) of pain as our reference, we included patients who scored between 4 and 7 on the VAS scale⁽²¹⁾. We only extracted teeth for patients who were suffering from unrestorable hopeless conditions and complaining of acute or chronic pulpitis. The time of salivary collection was another crucial factor that has been reflected in our inclusion and exclusion criteria; the circadian rhythm of cortisol had been proven to have an influential effect on the cortisol concentration in the body. The diurnal variation of plasma cortisol levels reaching peak values around 08:00 am and lower values around 24:00 pm, so we intended to collect saliva from the patients within the same time frame⁽²²⁾, to avoid any disturbance that circadian nature of cortisol can induce on our collected samples.

The patients were asked to chew on a cotton roll to stimulate salivary secretion (we avoided the use of citrus acid based salivary stimulants such as lemon because they have the potential to change salivary cortisol concentration which may be reflected on our sample accuracy)⁽²³⁾. We collected 3 salivary samples from each patient; the first sample was collected from the patient 10 minutes prior to local anesthetic injection and 5 minutes after the patient had been seated and get acquainted with clinical environment (sample 1), second sample was collected 6-8 minutes after local anesthetic injection (if the patient

required more than single injection another sample should be collected after 3-4 minutes after the second injection) (sample 2), the collection time of the final sample was performed 10-15 minutes post-extraction (sample 3).

The samples were collected and sent for analysis and interpretation, (they were subdivided into 3 samples, the first sample was the pre-extraction samples, second sample was the peri-extraction samples while the third sample represents the post-extraction samples) Tabel-1 ,which had been carried out at the clinical chemistry laboratories, college of dentistry, Al-Mustansiria University.

The analysis employed assay that employs the competitive inhibition enzyme immunoassay technique using Cortisol ELISA kit-BioCheck, Inc., Foster City, CA, USA. Immunoassay was used to measure an anti-antibody specific to the antibody of cortisol has been pre-coated onto a microplate. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for cortisol 3 and Horseradish Peroxidase (HRP) conjugated cortisol, and then incubated. A competitive inhibition reaction is launched between cortisol (Standards or samples) and Horseradish Peroxidase (HRP) conjugated cortisol with the antibody specific for cortisol. The more the amount of cortisol in samples, the fewer antibodies bound by Horseradish Peroxidase (HRP) conjugated cortisol. The substrate solutions are added to the wells, respectively. And the color develops in opposite to the amount of cortisol in the sample. The color development is stopped and the intensity of the color is measured (Figure-2).

Statistical analysis was performed using SPSS statistical software (versio19).ANOVA

and t-test was applied to obtain the significance and correlation between the examined groups.

Results

There is a significant variation that is well observed between all the three samples, which is highlighted inTable-2, and a highly significant variation was noticed in between the examined samples with a value of ($p=0.002$).

The mean value in the third sample (post-extraction salivary sample) was considerably high when compared to the other samples, which correlates with the patient's level of stress. There is a low variance between the patients in each sample; however, the highest value was observed in sample 1. The percentage of cortisol concentration of readings, 50%, 75%, and 90% were measured respectively (Table-2), the cortisol concentration was noticeably high in sample three throughout all the measured concentrations; specifically in the 90% third sample which was 0.518 $\mu\text{g}/\text{dl}$ which is remarkably high when compared to the first and second samples.

There is a positive correlation between the observed cum probe and expected cum probe in the entire three samples (fig.4, 3 and 5)

Discussion

Dental anxiety has been ranked fifth among commonly feared situations and that will affect the patient obedience to treatment, avoid dental visits, and anxiety usually generates stress that can create significant problems especially for those who are medically compromised (24-26).

The saliva is an excellent reservoir for cortisol and can be used as a diagnostic method for cortisol assessment (28). Salivary cortisol makes

up about 50 to 60% of the free cortisol in the plasma many reports have been shown that salivary cortisol is an accurate measure of adrenocortical function. The advantage of the saliva is the ease of collection in a non-invasive manner but because of its low concentration in saliva, it must be determined by a sensitive method such as RIA(Radio Immuno Assay); However, ELISA inhibition competitive immunoassay was widely used as well, and it can be considered as accurate as the RIA^(29,30). Our research tackled the relation of stress to dental extraction by measuring the cortisol levels in saliva; our study adopted the use of ELISA immunoassay for sample analysis and interpretation.

The correlation between cortisol levels and stress is related mainly to the anticipation of physical harm; people generally showed increased cortisol levels when they participate or exposed to a potentially harmful situations or when they feel threaten⁽³¹⁾, this basic instinct is reflected into dentistry in a dramatic way because the majority of patients attending dental clinics have some degree of anxiety and stress, particularly when they are undergoing dental extraction^(11, 12, 24).

The majority of dental treatments can have an influence on patient's anxiety and stress levels; however, dental extraction is shown to be the most stress related procedure when compared to other dental treatments. In a study conducted by Miller's et al (1995) it had been shown that extraction can have a major influence on the adrenal stress which is confirmed by measuring the levels of cortisol in saliva⁽¹¹⁾.

The study we conducted entitled to deal with the timing of the maximum stress during the course of dental extraction, many studies co-relate between the level of anxiety and the

pain perceived by patients during treatment. Okawa et al (2005) showed that anxiety can have vivid effect on pain perception during different stages of the extraction process such as local anesthetic injections and the extraction process itself⁽²⁷⁾. This high correlation between anxiety, stress and pain perception can have negative influence on the patient pain sensitivity because the escalation in the anxiety levels will cause changes in the pain perception which will affect the patient, dentist and quality of work.

We measured the level of cortisol, in three phases the first was pre-surgical, the second was peri-surgical and finally post-surgical phase. Anxiety and stress can be provoked before dental extraction is carried on and that suggests that the psychological perception of dental extraction as a potential harmful situation will provoke the mental element of stress⁽³²⁾, hence, the assessment of pre-operative anxiety which has been confirmed can have an influence on pain perception, our results showed that pre-extraction phase has a significant increase in cortisol concentration which can be correlated to the increased level of stress and anxiety. Lago-Mendez et al (2006). Confirmed that patient's anxiety increased before the extraction process and they validated their results depending on different types of anxiety scales. In our study we didn't notice any significant differences in cortisol levels between male and female patients and we assumed that the adrenal cortisol secretion in relation to stress is not influenced by gender but it is influenced by the degree of anxiety; however, Garip et al assessed the pre-surgical anxiety phase for patients undergoing surgical extraction under local anesthesia in Turkish population, they used two different anxiety scales

(Amsterdam preoperative anxiety and information scale (APAIS) and state trait anxiety inventory (STAI)), although the level of anxiety was significantly pronounced in almost all the participants; however, they also found that women expressed more anxious behavior than men involved in their study⁽³³⁾.

Although local anesthesia is considered as the main technique that is used to control pain during routine dental extraction, it can have many drawbacks such as inflicting pain and trauma; however, what falls into the scope of this study is the relation between local anesthetic injection and the provoking of stress. Patients receiving dental injections showed a significant increase in pain when they were highly anxious and vice versa patients anxiety levels increased when they received local anesthetic injections. Van et al. examined 247 patients undergoing invasive dental procedures, they assessed the relation between the level of anxiety and pain in relation to dental injections, they observed that highly anxious patients were more prone to pain and expressed longer pain perception after the administration of local anesthetic injection, they also noticed a gender variation; women anxiety level were higher than men participated in the study^(34, 35). The saliva we collected in our second phase of the study which was 6-8 minutes after the delivery of local anesthetic injection showed a significant increase in the cortisol levels, and we did not find any gender variation in our study.

The difference in salivary cortisol concentration between sample 1 and sample 2 was significantly observed in our study, sample 2 showed highly expressed cortisol levels when compared to sample 1, that can be due the additive stress that local anesthetic injection induced.

In our study sample 3 (post extraction) showed a significant increase in cortisol levels compared to sample 1 and 2, however we demonstrated that cortisol levels.

The study we conducted showed that the cortisol level was increasing from sample 1 – sample 3, which was significantly obvious in our statistical results, the variation between level of stress from samples 1, 2, and 3 can play a major role on levels of cortisol.

Conclusion

The positive correlation between the stress, anxiety and cortisol is well observed and documented. The examination of a sample from the Iraqi population revealed that salivary cortisol concentration is in its maximum level after the dental extraction procedure. Additional studies in the same population and analysis of the salivary cortisol during surgical operations (such as surgical removal of third molars), may have an influence on the stress level, hence the cortisol concentration; we assume that the cortisol concentration may increase in the first group (pre-extraction group) if the patients were undergoing surgical extraction due to the psychological perception of pain and the pre-surgical anticipation of complications, which require further analysis to confirm our assumptions. The measurements of cortisol in other dental branches can also carry different outcomes which require further investigations as well. Future work will include increasing the sample size and the addition of other anxiolytics modalities with the comparison between the effect of sedative drugs and cortisol level which will enhance our understanding of stress and how we can alter our treatment course in relation to the time frame during which

the patients will exhibit the highest cortisol levels, hence the highest stress.

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Case History (Cortisol)

Sex (gender): Male; Female;

1. Pregnant
 2. Contraceptive pills

Age/year:

Social history:
 Smoking;
 Alcohol;

Occupation:-----

Marital status:
 Married; Single; Divorced;

Medical history: -----
 If controlled: -----
 Types of drugs: -----

Drugs consumed on day of collection: -----

Time of admission: ----- [When the patient came to hospital]

Collection of samples:
 1- Pre – Extraction: ----- [Before L.A injection 10 min.]
 2- Peri – Extraction: ----- [Post. L.A 6-8 min.] «if more than
 single injection re-collect 3-4min. after 2nd injection»
 3- Post – Extraction: ----- [10-15 min. after extraction]

Pain VAS

Figure-1: case history form. Containing the inclusion and exclusion criteria

Table-I: Samples of saliva collected from each patient

Sample name	Time
1 st sample	10 minutes prior to L.A injection
2 nd sample	6-8 minutes after L.A injection and before extraction
3 rd sample	10-15 minutes after extraction

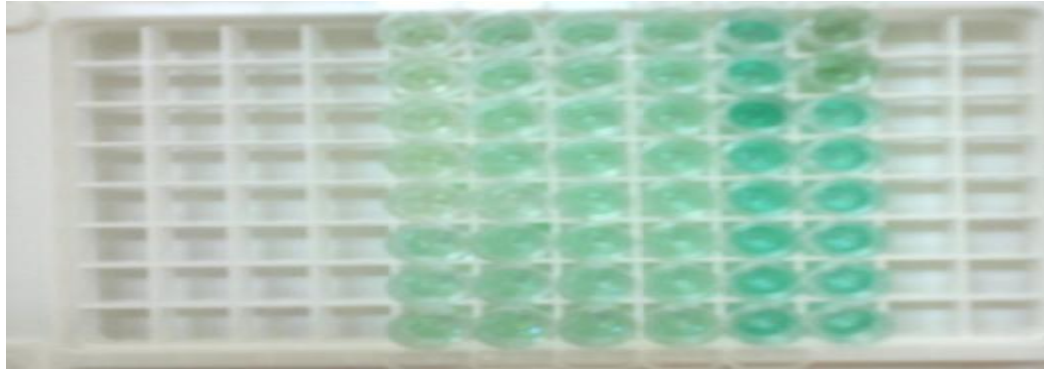
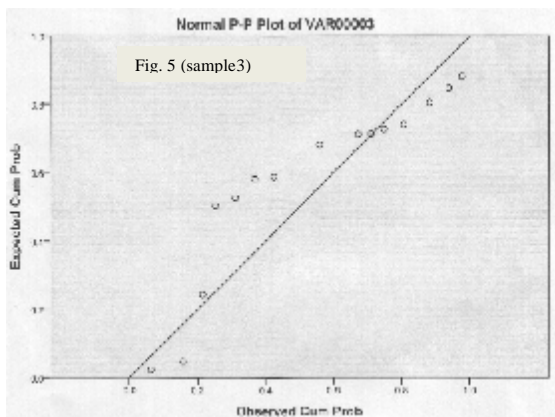
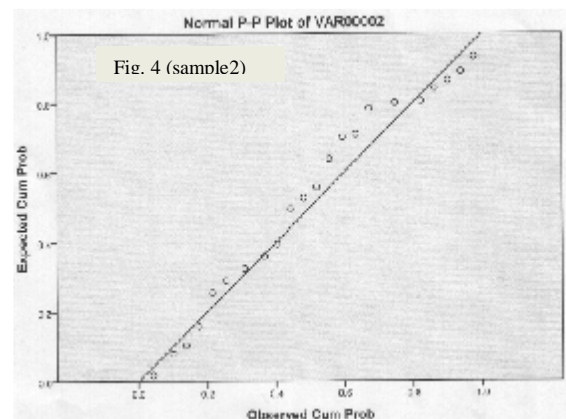
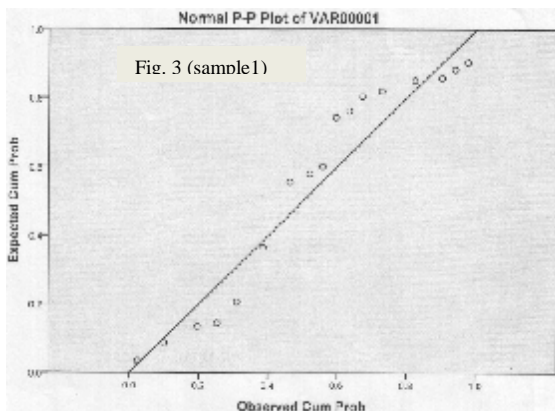


Figure -2 Microwells of ELISA estimation.

Table-2: Sample one (Pre – Extraction cortisol level), Sample two (Peri – Extraction cortisol level), Sample three (Post – Extraction cortisol level)

Function	First sample	Second sample	Third sample
NO. of samples	26	26	26
Mean ± SD	0.184± 0.184	0.307 ± 0.121	0.357 ± 0.141
STD. , Error of mean	0.036	0.024	0.028
Variance	0.034	0.015	0.020
%	50%	0.105 µg/dl	0.295 µg/dl
	75%	0.348 µg/dl	0.410 µg/dl
	90%	0.443 µg/dl	0.468 µg/dl
P≤ 0.05 between the groups	0.002		



Figures (3,4, and 5) The three figures are showing the expected statistical cum probe and the observed cum probe which reflect the positive correlation between the three groups which is significantly observed in the 3rd group