Research article

Salivary cortisol level pre and post administration of xylocaine hydrochloride local anesthesia for tooth extraction

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ABSTRACT

Aim: The study aims to compare the levels of cortisol in saliva before and after the administration of infiltration local anesthesia for tooth extraction and to estimate any possible correlation between dental anxiety and salivary cortisol level.

Materials and Methods: A total of 26 patients (18 female, 8 male) who need tooth extraction under infiltration local anesthesia were examined in two situations (pre anesthesia and post local anesthetic administration). Pre-local anesthetic: include twenty six patients before the administration of local anesthesia. Post local anesthetic : include twenty six patients after administration of local anesthesia. For each patients salivary sample has been collected by salivette to estimate the level of cortisol by using ELISA Kits, for all patients the anxiety levels has been measured before the administration of local anesthesia by using Modified Dental Anxiety Scale(MDAS).

Results: the results showed no significant differences between salivary cortisol for patients before the administration of local anesthesia (19.20±4.39)ng/ml and after the administration of local anesthesia (20.48±4.77)ng/ml   with p.0.05, the results also showed that the anxiety levels measured by MDAS was (12.00±2.4587) with no significant correlation between the anxiety level and salivary cortisol levels.

Conclusions: : tooth extraction and administration of infiltration local anesthesia will not cause significant effect on salivary cortisol levels, also this study showed that there was no significant correlation between anxiety levels measured by MDAS and cortisol levels during dental treatments(tooth extraction). **Copyright © acascipub.com, all rights reserved.**

Key Words: salivary cortisol, local anesthesia, dental anxiety

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INTRODUCTION

Dental treatment may cause pain and discomfort, even the expectation of pain increase dental anxiety, thus maintaining the number of dentally anxious person [1].
Dental anxiety is the feeling of tension associated with dental treatment and is not necessarily connected to external stimuli, while dental fear is defined as a specific anxiety , that it is the predisposition for a negative experience in the dental surgery [2] dental anxiety and fear pose a significant problem in patient management , with anxious patients more likely to avoid or delay treatment and more likely to cancel dental appointments [3,4,5] The interpretation of a situation as being stressful leads to activation of the hypothalamic pituitary – adrenal (HPA) axis and to the ultimate secretion of cortisol and catecholamines in human . the end product of HPS activation (cortisol and catecholamine)are easily measurable in blood , urine and saliva , chronic stress is associated with the activation of (HPA) axis (measured by salivary cortisol) [6], the wide spread use of questionnaires and behavioral measures for assessing dental anxiety or fear is a sign of the ready adoption and application of psychological method to the study of oral health [7].

Aims of the study: this study aims to compare the levels of cortisol in saliva before and after the administration of infiltration local anesthesia for tooth extraction and to estimate any possible correlation between dental anxiety and salivary cortisol level.

Patients, Materials and Methods

This study was carried out at the specialized center for dentistry in Mosul city / Iraq, from February 2013 to July 2013. A total number of   patients included in this study were (26) patients with an average age between (20- 40) year old

Criteria of patients selection: all patients should required infiltration LA injection for dental treatment (extraction ,)for maxillary teeth only, no history of compromised medical status , no recent use of antibiotics or analgesic drugs (in the last one week), non-pregnant or lactating females, non- smoker, non – alcoholic, and agreed to participate in this study and signed the consent form.

Study design and sample

1- **patient grouping:** patients included in this study were examined in two main situations:

Pre- anesthetic situation :included patients who were required a dental treatment (tooth extraction)and were seated on a dental chair for dental and clinical examination **but before taking LA injection** , this group consist of a total number of (26) patients (8 M, 18   F), their mean of age (20-40   ) years.

Post- anesthetic situation : included patients who were   **taking LA**

 injection   for dental treatments (tooth extraction), this group consist of a total number of (26) patients (8   M, 18   F), their mean of age (20-40) year**.**

2- **drug used**
Local anesthesia:

Local anesthetic cartridge 1.8 ml (lidocaine –hamein 2% with 1:80,000 adrenalin) MOH/ IRAQ used to produce anesthesia by infiltration to maxillary teeth[8] , the anesthesia were administrate by the researcher only for those patients.

Salivary sample collection and storage:

Material and equipment used for collection, separation and storage of saliva

Equipment	supplier
Salivette	Sarstedt(Germany)
Centrifuge 80-1 TAPLETOP low speed	GSJ, China
Micro pipette 10-100 ml and tips	Jianan, China

Eppendroff tube 1.5 ml	China
Rank for Eppendroff tube	China
Refrigerator	Biofamily, Korea
Freezer	Universal ,Korea
Cool box	China

Salivary sample collection and storage:

Unstimulated saliva were collected from patients in before and after and administration of local anesthesia by 5-7 minute by asking the patients to rinse his or her mouth with 10 ml of tap water to remove food debris then Cotton-based techniques include using a simple cotton dental roll of specialized devices (Salivette) , a cotton roll is sucked or chewed in patients mouth (cheek , floor of mouth ,and over the tongue for a 1 minute, this allow s the saliva to be absorbed and collected in the swab in an easy and hygienic fashion,all sample should be collected between 9 -11 am and should be clear of blood contamination[9]. Salivette then centrifuged at 3000 rpm for 10 min , which yield a clear fluid sample at the bottom of tube after centrifuge while leaving debris and residue in cotton swab , then the clear fluid place in sterile eppendroff tube and stored at deep freeze _20 Ċ to be thawed for analysis [10].

Anxiety level measurement:

Anxiety level were measured according to modified dental anxiety scale MDAS [11]   . This consist of five multiple choice items , each with five responses. The cut- off point used was a score ≥16 , indicating that the patient felt somewhat anxious about dental treatment . a score of 19 or more indicated a highly anxious patient [9].Figure-1-

The anxiety was measured for patients before taking local anesthetic only in the reception room, and the form was filled by the researcher herself by interviewing each patient separately.

1. If you went to your Dentist for TREATMENT TOMORROW, how would you feel?

Not	*Slightly*	*Fairly*	*Very*	*Extremely*
Anxious ☐	*Anxious* ☐	*Anxious* ☐	*Anxious* ☐	*Anxious* ☐

2. If you were sitting in the WAITING ROOM (waiting for treatment), how would you feel?

Not	*Slightly*	*Fairly*	*Very*	**Extremely**
Anxious ☐	*Anxious* ☐	*Anxious* ☐	*Anxious* ☐	*Anxious* ☐

3. If you were about to have a TOOTH DRILLED, how would you feel?

Not	*Slightly*	*Fairly*	*Very*	**Extremely**
Anxious ☐	*Anxious* ☐	*Anxious* ☐	*Anxious* ☐	*Anxious* ☐

4. If you were about to have your TEETH SCALED AND POLISHED, how would you feel?

Not	*Slightly*	*Fairly*	*Very*	**Extremely**
Anxious ☐	*Anxious* ☐	*Anxious* ☐	*Anxious* ☐	*Anxious* ☐

5. **If you were about to have a LOCAL ANAESTHETIC INJECTION in your gum, above an upper back tooth, how would you feel?**

Not	*Slightly*	*Fairly*	*Very*	**Extremely**
Anxious ☐	**Anxious** ☐	**Anxious** ☐	**Anxious** ☐	**Anxious** ☐

1 = Not anxious

2 = Slightly anxious

3 = Fairly anxious

4 = Very anxious

5 = Extremely anxious

Total score is a sum of all five items, range 5 to 25:

Figure 1: The modified dental anxiety scale

Salivary cortisol measurements:

Materials:

ELISA,LT-400MS Reader	Labtech ,UK
LT -300 Microplate strip automatic washer	Labtech ,UK
Human Cortisol ELISA Kit	MyBioSource ,USA
Micropipette 100µ L and 1000 µ L , multi channel micropipette 300 µ L , and tips	China
Deionized water	NDI, Iraq
37 c incubater	china

Materials supplied in the kit:

1	Standard (240ng/ml)	0.5 ml	7	Chromogranin solution A	6 ml
2	Standard diluent	3 ml	8	Chromogranin solution B	6 ml
3	Microelisa	12w*8s	9	Stop solution	6 ml

	stripplate				
4	Str-HRP-Conjugate Reagent	6 ml	10	**Instruction**	**1**
5	30*wash solution	20 ml	11	Closure plate membrane	2
6	Biotin-CORTISOL-Ab	1 ml	12	Sealed bag	1

Methods:

Suggested in this study and the test were performed and interpreted following instruction out lined in kit

Principle of the test

The kit uses a double-antibody sandwich enzyme-linked immunosorbent Ass ay (ELISA) to assay the level of Human Cortisol (CORTISOL)in saliva. Human Substance Cortisol (CORTISOL)of saliva were positively correlated

Assay procedure:

1. Standard dilution : this test kit will supply one original Standard reagent and dilution occur according to the instruction

120ng/ml	Standard No.5	120µl Original Standard +120µl Standard diluent
60ng/ml	Standard No.4	120µl Standard No. 5+120µl Standard diluent
30ng/ml	Standard No.3	+120µl 120µl Standard No.4 Standard diluent
15ng/ml	Standard No.2	120µl Standard No.3 +120µl Standard diluent
7.5ng/ml	Standard No.1	120µl Standard No.2 +120µl Standard diluent

240ng/ml 120 ng/ml 60 ng/ml 30ng/ml 15 ng/ml 7.5 ng/ml

2.The quantity of the plates depends on the quantities of to-be-tested saliva and the standards. .

3.Inject saliva:

Blank well: don't add saliva and CORTISOL –antibody labeled with biotin, Streptavidin-HRP, only Chromogen solution A and B, and stop solution are allowed; other operations are the same.

Standard wells: add standard 50μl, Streptavidin-HRP 50μl (since the standard already has combined biotin antibody, it is not necessary to add the antibody); To be test wells: add saliva 40μl, and then add both CORTISOL -antibody 10μl and Streptavidin-HRP 50μl. Then seal the sealing memberance, and gently shaking, incubated 60 minutes at 37 .

4.Confection: dilute 30 times the 30×washing concentrate with distilled water as standby.

5. Washing: remove the memberance carefully, and drain the liquid, shake away the remaining water.

6. Add chromogen solution A 50μl, then chromogen solution B 50μl to each well. Gently mixed, incubate for 10 min at 37 away from light.

7. Stop: Add Stop Solution 50μl into each well to stop the reaction (the blue changes into yellow immediately).

8. Final measurement: Take blank well as zero , measure the optical density (OD) under 450 nm wavelength which should be carried out within 15min after adding

9. According to standards' concentration and the corresponding OD values, calculate out the standard curve linear regression equation, and then apply the OD values of the saliva on the regression equation to calculate the corresponding saliva's concentration. .

$y = 54.693x - 10.774$
$R^2 = 0.9909$
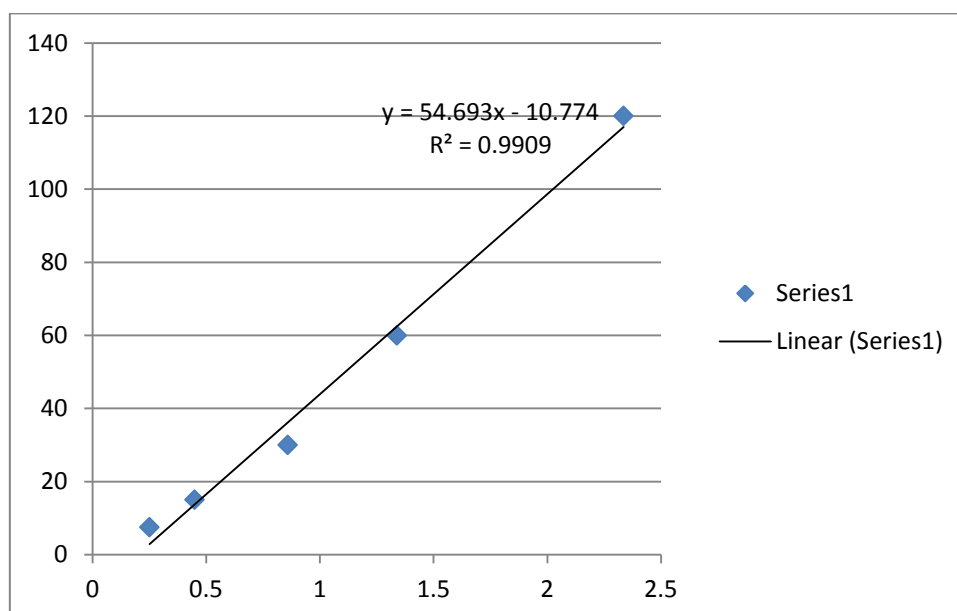
Data Analysis:

The data obtained from this study were subjected to the statistical analysis include descriptive and analytic methods. For descriptive way the mean of variance were used, while one way Analysis of variance and Duncan's Test for (intra – group comparision) and for (inter – group) matching with 5% level of significance, paired t-test had been used to compare between the studying group, and Kruskal Wallis test was used to determine the mean value of MDAS while pearson correlation test and spearman test was used to estimate the possible correlation between the studying parameters.

RESULTS

A total of 26 patients participated in this study. Most of them were females (69.2% females , 30.8% males) Table no.1 The mean age of samples was 22.5 ± 6.68 years for female and 30.5 ± 3.5 years for male with age range 20-40 years Table 2.

Table 1: Distribution of the study samples

sex	number	Percentage	P-value	DF	χ^2
Male	**8**	**(30.8)**			
Female	**18**	**(69.2)**			
Total	26	100	0.7	**2**	**0.556**

Analysis was performed by ANOVA test

Table 2: Descriptive statistic of study samples for both sexes

Sex	Mean of age ± SD	Minim	Maximum
Females	22.5 ± 6.68	20	40
males	30.5 ± 3.5	20	40

SD: standard deviation

The results of this study which performed by paired t-test showed that the mean amount of cortisol in for patients before taking local anesthesia was(19.20±4.39) ng/ml while its amount was(20.48±4.77) ng/ml for patients after taking local anesthesia with no significant differences between them (p.0.05) Table 3.

Table 3: Cortisol measurement pre and post – anesthetic administration

Parameters	Pre- local anesthetic Mean±SD	Post- local anesthetic Mean+SD	Difference Mean±SD	95% CI of difference	T-value	DF	P-value
Cortisol ng/ml	19.20±4.39	20.48±4.77	-0.17±1.39	-0.83 -0.28	-1.010	25	0.3

Analysis were performed by the use of paired t-test

The results of this study showed the measurement of MDAS for patients before administration of local anesthesia which performed by Kruskal Wallis test which demonstrate that the mean value was(12.00±2.4587) in patients undergo extraction of teeth .Table 4.

Table 4: MDAS measurement in

Parameter	Median±SD (min-max)
MDAS	12.00±2.4587 (-16)

Analysis were performed by the Kruskal Wallis tets

The pearson correlation test and spearman test results showed that there are no correlation exist between MDAS and cortisol measurement for patients undergo tooth extraction Table 5.

Table 5: Correlation between MDAS and cortisol [t]

parameter	MDAS
Cortisol	**0.23**
	0.2

Note : upper bold fig (correlation coefficient r), lower figure (p-value), analysis were performed by pearson correlation test and spearman test

DISCUSSION

This study aims to compare the salivary cortisol levels before and after the administration of infiltration local anesthesia for tooth extraction and to found the possible correlation between the salivary cortisol and dental anxiety. The results of this study showed no significant differences between the mean amount of cortisol before and after administration of local anesthesia this results was disagreements with the study of Chamani etal (2006) which showed that cortisol decreased after administration of local anesthesia for tooth extraction [12] As in a previous study by Kruger etal (2005) which found that there is no statistic differences found between salivary cortisol levels[13]. The sympathetic nervous system activity is a suitable standard for the study of biological foundation of emotions. One of the investigating ways for system activity could be the cortisol level measurements [14]. Salivary cortisol levels have been frequently used as an objective measure of stress in psychological studies [15] the accessibility of saliva and the non invasive manner obtaining the specimen are further advantages of using saliva as a diagnosis tools [16]. A few studies have measured physiological stress during dental procedure. Miller et al(1995) have shown that the saliva cortisol decreased from the initial levels to the end of procedures by a bout 15%of patients undergo different treatments . they also showed that adrenal stress respond associated with tooth extraction is greater than that of associated with other routine dental procedures[17]

The possible explanation of the results of this study is that dental anxiety alone was not enough to cause a reaction in HPA axis and to produce a significant change in salivary cortisol concentration. In the majority of cases , dental emergencies are not life threatening , and patients do not go to the dentist immediately upon recognizing a dental problem . as these patients may avoid receiving the required dental care , a delayed dental visit can decrease the output of cortisol as a results of negative feedback on the HPA axis[9] The anxiety was assisted in this study by using MDAS , it is a brief 5 item questionnaire with a consistent answering scheme for each item ranging from ' not anxious ' to ' extremely anxious', it does not increase patients fears when completed [18,19] completion of the questionnaires can significantly reduce state anxiety in the practice setting [20]. It has a good psychometric properties, is relatively quick to complete and scoring is easy[21].

The results of this study showed that there are no significant correlation between the MDAS and cortisol levels this was in agreement with the study of Kanegane etal (2009)[9,] and Sadi etal (2013)[22] while it is disagreement with the study of Benjamins etal (1992)which found that salivary cortisol were significantly elevated if patients manifested anxiety in sever dental stress situations[23]. Although pain may occur during dental treatment, not everyone develops dental anxiety. An aversive experience may contribute , but particular psychological traits should also be associated[24] Ooternik etal (2008) found that individuals who had been exposed to distressing experience in the past reported high level of dental anxiety and other more distressing experiences, dental anxiety alone was not enough to cause a reaction in HPA and to produce a significant change in salivary cortisol concentrations[25] A suggestion for future studying is to find a possible correlation between blood and salivary cortisol levels before and after administration of local anesthesia and the effects of pre treatments methods which have been used to reduce dental anxiety on such parameters.

Conclusion

Tooth extraction and administration of infiltration local anesthesia(lidocaine hydrochloride2%+1.80,000 adrenalin) will not cause significant effect on salivary cortisol levels, also this study showed that there was no significant correlation between anxiety levels measured by MDAS and cortisol levels during dental treatments(tooth extraction).

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