EVALUATION OF DENTAL ANXIETY IN CHILDREN BY ESTIMATING THE SALIVARY CORTISOL LEVELS BEFORE AND AFTER DENTAL PROCEDURES

Running title: To evaluate dental anxiety by estimating the salivary cortisol levels.

ABSTRACT

Aim: Dental procedures in general range from simple examination to complex stressful procedures like extractions. This can cause varied levels of anxiety in children based on the severity and duration of the procedure. This study was designed with the aim to assess changes in salivary cortisol levels due to the various dental procedures carried out in children. **Materials and Methods:** The sample consisted of a total of 30 children of the 5-9 age group whose parents consented to examination and further treatment. Children who had been given a Frankl score of positive (+) or definitely positive (++) upon initial examination were only included in the study. Children with negative (-) and definitely negative (--) Frankl scores, any history of systemic diseases, on corticosteroid therapy, having fever, or medically compromised were excluded from the study. Unstimulated saliva was collected before and after the procedure and was analysed using ELISA. The data collected was statistically analysed and assessed for significance. **Results:** Out of the 30 children 17 were boys and 13 were girls and the difference was statistically insignificant. There was no significant difference in age between the genders (P = 0.12). Paired t - test was done to analyse the intra group data and ANOVA test was done to compare between the groups. Compared to the three procedures restorative was least stressful whereas extractions were most stressful and this result was statistically significant. **Conclusions:** Within the limitations of this study, we can conclude that salivary cortisol is a good indicator of acute stress. Extraction procedures seem to be the most stressful of all the procedures.

Keywords: Salivary cortisol levels; Dental anxiety; Psychological stress; Dental pain

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1. INTRODUCTION:

Stress is defined as a nonspecific reaction of the body to any request for adjustment or adaptation, performed in a stereotyped manner on the basis of identical biochemical changes [1]. Anxiety is one of the many symptoms of the reaction of human beings to stress situations [2]. Homeostasis in the oral environment is the most important condition for maintaining oral health. Imbalance that can occur with all mentioned above variables affects the oral environment [3,4].

Cortisol, known as a stress hormone, is the most potent glucocorticoid, participates in the response of the body to stress conditions and enters into complex interactions with the hormonal and immune system known as hypothalamus pituitary- adrenal axis [5–8].Cortisol has a diurnal variation, and cortisol secretory activity is characterized by peak levels 20–30 min after awakening and a declining pattern thereafter [9–11]. The diurnal rhythm of cortisol is a robust rhythm and is mainly unaffected by age, gender, or pubertal status [12–14]. Salivary cortisol is known to be an indicator of the concentration of unbound cortisol in serum [15]. Through specific receptors, has a major impact on the intermediary metabolism and has major anti-inflammatory, immunosuppressive and antiphlogistic properties [16]. Cortisol release has an ACTH (pituitary adrenocorticotropic hormone) - dependent circadian rhythm [17].

Saliva is a natural ultrafiltrate of blood. Steroids, such as cortisol, not bound by carrier protein, diffuse freely into saliva. Since the concentration of carrier proteins in saliva is extremely low, measurements in saliva for the most part represent the free fraction of the hormone [18]. Transfer of cortisol from the blood to the saliva occurs by passive diffusion [19,20] and therefore, the concentration of cortisol in the saliva does not depend on the salivary flow rate.

With advances in microbiology, immunology and biochemistry, salivary testing in clinical and research settings is rapidly proving to be a practical and reliable means of recognizing oral signs of systemic illness and exposure to risk factors. The components of saliva act as a "mirror of the body' health" and the widespread use and growing acceptability of saliva as a diagnostic tool is helping individuals , researchers, health care professionals and community health programs to better detect, monitor disease and to improve the general health of the public [21–23].

Collection of serum for cortisol analysis is stressful, thereby directly elevating free cortisol concentration and distorting the results of the tests [24,25]. Increasing interest in salivary cortisol assessment is due to a number of significant advantages: stress-free ease collection, the absence of special handling or storage procedures, the correlation with cortisol levels in blood, not depending on flow rate, smaller sample aliquots, the possibility of a dynamic study, greater sensitivity and non- invasive collection procedure and a good cooperation with patients. Salivary cortisol measurement would appear to be the measurement of choice in human stress where individual stress factors are to be identified and studied [18,22,23].

The elevation of salivary cortisol levels can be the result of both physiological and psychological stress. The acute physic stress (hypoglycemia) the psychologic acute stress (acute anxiety) induces the secretion of ACTH and rise in salivary cortisol [17,26,27]. Salivary cortisol was also found to be increased among children due to their anticipation of surgical procedure including

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dental procedures [28]. Evidence is available that cortisol exhibits higher levels in abused children, children raised outside the family environment, predisposed to obesity, diabetes and etc [29,30].

Dental procedures in general range from simple examination to complex stressful procedures like extractions. This can cause varied responses in children based on the severity and duration of the procedure. Previous studies have tested for an increase in salivary cortisol levels in relation to dental anxiety but not much study has been conducted to separately study the effect of the various procedures.

Our department is passionate about child care, we have published numerous high quality articles in this domain over the past 3 years [31–51]. With this inspiration we planned to pursue research on evaluation of dental anxiety in children by estimating the salivary cortisol levels before and after dental procedures.This study was designed and carried out with the aim to assess changes in salivary cortisol levels due to stress due to the various dental procedures carried out in children. Objectives of the study were to find out if any correlation exists between severity and duration of procedure in relation to stress and the salivary cortisol to evaluate which step in a dental procedure is the most stressful.

2. MATERIALS AND METHODS:

2.1 Ethical approval

The study was registered with the Institutional Review Board of the Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India. Ethical approval was obtained from the Institutional Review Board of the SIMATS. Informed consent was obtained from all parents of the children before including them in the study.

2.2 Source of patients

The sample comprised a total of 30 children of the 5-9 age group whose parents consented to examination and further treatment. Children who had been given a Frankl score of positive (+) or definitely positive (++) upon initial examination were only included in the study. Children with negative (-) and definitely negative (--) Frankl scores, any history of systemic diseases, on corticosteroid therapy, having fever, or medically compromised were excluded from the study. The children were divided into three groups:

Group A – Comprised of patients who underwent restorative procedures.

Group B – Comprised of patients who underwent pulpectomy.

Group C – Comprised of patients who underwent extractions.

2.3 Clinical procedure

A single operator carried out a standard dental examination and the treatment for the 30 children during a morning time between 9 and 11am. None of the children were sedated during the treatment or dental examination.After routine dental examination (including hard and soft tissue examination and radiograph as needed) the required treatment was carried out.

The restorative procedure involving excavation of caries with high speed handpiece and placement of glass ionomer restorative material(Type 2) for the class I cavity was done on a molar teeth. Children indicated for extraction and pulpectomy were first injected with the local anesthetic which was 2% lidocaine hydrochloride with 1:100,000 epinephrine. The pulpectomy

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was done by access opening using a high speed handpiece followed by extirpation of pulp and consecutive cleaning and shaping of the root canals using hand k- files and rotary kedo files followed by obturation and entrance filling using glass ionomer restorative material (Type 2).

2.4 Collection and analysis of saliva

Unstimulated saliva, about 1-2 ml was collected from the child after rinsing the mouth by half glass of a tap water at two intervals, as soon as the child sits in the dental chair and 30 min after the completion of the procedure.

Unstimulated saliva was collected by asking the patient to expectorate into a disposable plastic container.. The samples from two children undergoing dental extraction were contaminated with blood. These blood contaminated samples were excluded in the study because of likelihood of contamination with plasma cortisol.

The disposable plastic containers containing the saliva samples were tightly closed and were stored at - 20°C soon after collection till the samples had reached the laboratory. Total 60 samples were collected to determine salivary cortisol levels using the ELISA method with Saliva Cortisol Enzyme Immunoassay Kit, USA and the readings were recorded.

2.5 Statistical Analyses

The collected data was tabulated into Microsoft office Excel 2013 transferred to SPSS version 26.0 software (SPSS, Chicago, IL, USA) for statistical analysis. This data was analyzed statistically using the paired t-test, and ANOVA to evaluate differences in salivary cortisol levels, before and after subjection of a stressor, between the various groups to find the significance among various parameters. The adaptability of the children to the stressor in between the groups was evaluated by comparing the post-treatment salivary cortisol levels.

3. RESULTS AND DISCUSSION:

Out of the 30 children 17 were boys and 13 were girls with a mean age of 6.59. The patients were aged between 5 and 9 years, there was no significant difference in age between the genders (P = 0.12). Although there were more males than females, the difference was found to be statistically insignificant.

From the 60 samples taken the average salivary cortisol levels irrespective of the procedure done was 2.12 before and 2.65 after the procedure and the result was statistically significant (p = 0.00) [Table 1].

Paired t-test was performed and there was statistical increase (P = 0.28) in salivary cortisol levels before and after procedure when restoration was done (Table 2), (P = 0.000) before and after procedure when extraction was done (Table 3), (P = 0.000) before and after procedure when pulpectomy was carried out (Table 4). The salivary cortisol levels varied significantly across groups. The group A had the lowest levels of salivary cortisol, while the patients in group C had the highest levels of salivary cortisol. (Table 5) Shows there was a significant increase in salivary cortisol levels by performing ANOVA among the 3 groups and found that the values were significant before (p = 0.002) and after (p = 0.001) the procedure.

Time of collection	**Number of patients**	**Salivary cortisol**	

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		levels (µg/dl) (Mean)	p - value
Before procedure	30	2.12	0.00
After procedure	30	2.65	

Table 1: Table comparing the overall salivary cortisol levels (µg/dl) before and after the procedure.

Time of collection	Number of patients	Salivary cortisol levels (µg/dl) (Mean)	p - value
Before procedure	10	1.89	0.28
After procedure	10	2.34	

Table 2: Table comparing the salivary cortisol levels (µg/dl) before and after restoration (Group A).

Time of collection	Number of patients	Salivary cortisol levels (µg/dl) (Mean)	p - value
Before procedure	10	2.22	0.00
After procedure	10	2.66	

Table 3: Table comparing the salivary cortisol levels (µg/dl) before and after pulpectomy (Group B).

Time of collection	Number of patients	Salivary cortisol	p - value

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		levels (µg/dl) (Mean)	
Before procedure	10	2.25	0.00
After procedure	10	2.94	

Table 4: Table comparing the salivary cortisol levels (µg/dl) before and after extractions (Group C).

Time of collection	**Salivary cortisol levels (µg/dl) (Mean)**			**p - value**
	Group A	**Group B**	**Group C**	
Before procedure	1.89	2.22	2.25	0.002
After procedure	2.34	2.66	2.94	0.001

Table 5: Table comparing the salivary cortisol levels (µg/dl) before and after procedure among the three groups.

Dental caries is an infectious and communicable disease. The disease is recognized to require various primary etiologic factors like host, a dietary substrate and aciduric bacteria. Many secondary factors, such as salivary composition and flow rate, oral hygiene and diet also influence the caries process [52].Child dental fear is one of the major problems that the dentist faces in practice and has been linked to poor dental health [53–55]. It was stated that children with stressful emotional states are more susceptible to infection with s mutans or other bacteria, due to immunosuppression related to stress including dental caries [24].

In previous studies the importance of circadian rhythm of cortisol was also emphasized [56]. Considering this the saliva samples were collected from children in the present study at the same appointment hours. All the appointments were scheduled for morning appointments, between 9:00-11:00 A.M. This helped us to study if pain, discomfort during eating, their limitation due to excessive dental decay and alleviation of these during treatment had a bearing on levels of salivary cortisol [25,57].

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The saliva samples were stored at - 20°C soon after collection till the samples have reached the laboratory. Previous studies have confirmed that salivary cortisol is stable at room temperature for 2-4 weeks. However the sampling devices will start to mold within 4-7 days at room temperature.as a result this produces a very bad smelling sample but does not appear to affect the cortisol levels .So whenever possible saliva samples are stored at -20°C or lower [15].

This study reported no difference in anxiety between girls and boys, a finding supported by previous studies [58–60]. This was contrary to other findings where more females than males were in the anxious group [61,62]. This study found no significant difference in age. The fact that this study found no age difference in fear among the different groups of this study seems to validate our rationale of choosing the age of 5–9 years as a homogenous study [63].

Cortisol levels in saliva for the group who received long and painful dental procedures is significantly higher than those who received a short and painless dental procedure. These data suggest that adrenal stress response associated with long and painful dental procedures is greater than that associated with short and painless dental procedures [64].

In the present study, salivary cortisol and anxiety levels were higher prior to dental treatment for children who were about to undergo extraction or pulpectomy as compared to restorations. The pain felt by the child due to pulpal inflammation or furcal involvement could be a reason for this increase. This could also be due to the fact that the child may be stressed in anticipation of what will happen after entering the dental examination room and being seated on the dental chair. The high level of stress causes an extreme increase in adrenal hyperactivity, which leads to increased secretion of cortisol level. Therefore, the increase in salivary cortisol level is related to the level of dental anxiety, this finding is in agreement with Benjamins et al [65].

Overall children undergoing restorative treatment had less dental anxiety. But it has been noted that few children had a higher cortisol level during restorative procedures. This could be related to the unpleasant sounds of air rotor along with suction and drilling for a longer period of time. This could have resulted in an increased level of anxiety, directly elevating the levels of salivary cortisol [66].

Comparatively extraction and pulpectomy procedures caused high dental anxiety and showed high salivary cortisol levels. This can be attributed to apprehension as a stimulus to adrenocortical secretion. This finding is based on the increase in the cortisol value at the point. Shannon et al., [67] have reported that the anxiety of local anesthesia administration and oral surgery can cause elevations in cortisol in patients undergoing dental procedures. In contrast Banks [68] and Franksson and Gemzell [66] have reported that preoperative apprehension is not a stimulus to corticosteroid secretion. Moreover the stress associated with extraction is greater than that associated with other dental procedures. This is in agreement with the above results. The physical forces and pressure asserted during an extraction could be one of the causes for the rise in the cortisol levels. It was found that the stress associated with extractions and pulpectomy persists into the postoperative period. Rise in salivary cortisol levels after 30 min of extraction may be suggestive of loss of a tooth generated fear in children, several studies support this finding and in fact elevated levels have been shown to remain elevated for about 4-7 h after surgery [68,69]. Only Hempenstall et al., [70] reported that plasma cortisol decreased during the period

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after dental surgery. However, most studies have concluded that significant cortisol increases are not seen before or during minor operations but are increased in the postoperative period which is similar to the findings in this study[68,70].

According to McCaul and Mallot [71], distracting the child from an unpleasant stimulus can result in decreased pain perception and thereby their anxiety. So modern behavior guidance techniques can be used to reduce the children's anxiety to LA injection and for other invasive procedures including non-pharmacological and pharmacological types (general anesthesia and conscious sedation) [72] like virtual reality distraction [73].

There was a source of bias present in the study as both the operator and the participant could not be blinded as the specific treatment done for each patient was invariably known.

4. CONCLUSION:

Within the limitations of this study, we can conclude that salivary cortisol is a good indicator of acute stress. Extraction procedures seem to be the most stressful of all the procedures. Stress associated with extraction and pulpectomy persists to a postoperative period. Therefore from this study we can conclude that usage of recent behaviour modification techniques can help reduce their anxiety levels and thereby provide a positive dental experience.

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