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EVALUATION OF CORTISOL CONCENTRATION IN SALIVA AS A MEASURE OF STRESS INPATIENTS HAVING ROUTINE DENTAL EXTRACTION

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ABSTRACT **Background and Objective:** The anxiety and fear are the major factors that regulate cortisol levels. This prospective study was conducted to estimate the salivary cortisol among patients subjected to dental extraction and correlate any existence between pre- and post-extraction cortisol levels. **Materials and Methods:** The study sample included 10 patients indicated for a dental extraction between the ages of 18 and 60 years. A total of 20 samples of saliva from 10 subjects before and after extraction were analysed. Saliva samples from the control group and pre- and post-extraction samples from the study group participants and vital parameters were collected. We used quantitative ELISA Kit, to estimate the salivary cortisol level. The descriptive statistics mean, S.d. was calculated. To compare the parameters between groups and within groups for normal data parametric Wilcoxon sign rank test was used among the study population. **Results:** The mean value of cortisol concentration of saliva was significantly greater in the post extraction group than pre extraction group. **Conclusion:** Patient's anticipation and anxiety toward dental therapy elevate the cortisol level. Dental surgeons should treat the patients more conveniently and effectively without any strains and provide a comfortable atmosphere to avoid stress-related consequences.

KEYWORDS : Cortisol, Saliva, Stress

INTRODUCTION:

Cortisol, a stress hormone, has been used as an indicator in the stress evaluation studies[1]. During period of active stress, cortisol promotes survival by mobilizing energy reserves as well as long terms stress related adaptive changes such as shaping and regulating a number of physiological processes, including immune responsiveness and activation of sympathetic nervous system.

Selye was the first to describe stress in the 1930s as the non-specific response of the body to any demand whether it is caused by physical and psychological stress affect the adrenal cortex results in, secretion of cortisol in stressful conditions[2]. To evaluate stress, and its salivary component has been used by dental research workers to measure stress during dental procedures[2].

Salivary cortisol concentrations have shown that routine dental and oral and maxillofacial surgery procedures cause stress[2]. For example, dental extraction is stressful and is one of the routine procedures done by dental surgeons in every day practice.[2]

In OMFS dental anxiety, a commonly feared situations that affect the patient obedience to treatment, avoid dental visits, and anxiety usually generates stress that create significant problems especially for those who are medically compromised. The saliva is an excellent reservoir and can be used as a diagnostic method for assessment. 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 immune assay was widely used as well, and it can be considered as accurate as the RIA.[3]

The measurement of salivary cortisol which provides a simple, non-invasive, and stress-free measure are frequently used in studies of the hypothalamic-pituitary-adrenal axis activity. e.g. in patients with psychiatric disorders and studies of effects of occupational stress[4]. Another advantage of measuring cortisol in saliva is the possibility to collect several samples within a short period of time and therefore measure reactivity and recovery of the HPA-axis. Because of the easy collection of saliva, e.g. using Salivette H tampons, samples maybe collected at home, at work, or elsewhere. Samples are often stored at room temperature (RT) or in the refrigerator until delivery to the laboratory.[5] Cortisol secretion is regulated by the HPA axis. The cortisol level in the peripheral blood is thus subject to a circadiarrhythm with the highest values (200–800nmol/L) in the early morning during the last hours of sleep, followed by a gradual decline in the course of the day, with the minimum level (300nmol/L) being reached in the early evening[6]. This rhythmic secretion can also be detected in the saliva and gingival crevicular fluid[6]. Thus we are performing this prospective study to evaluate the salivary cortisol concentration in saliva as a measure of stress of the patient undergoing routine dental extraction procedure.

MATERIALAND METHODS

This prospective study was conducted on 10 patients who had underwent extraction in the Department of Oral and Maxillofacial surgery over a period of one year. The eligibility criteria were patients in the age range 18-60 years who had to undergo single or multiple tooth forcep extraction in region of maxilla and mandible with the ASA class I. Exclusion Criteria includes any existing systemic disease or taking any drug that may affect parameters to be examined and patients with conditions of decreased or altered secretion of saliva. Measurement of cortisol in saliva is done for all the patients before and after the extraction. A total of 20 samples of saliva from 10 patients (study group) before and after extraction were collected. Saliva samples from the control group and vital parameters were also analysed. Data was collected through ELISAKIT. Ethical clearance was approved by the Institution Review Board(IRB) with no. ITSCDSR/IIEC/LD/OS/2019-22/003.

Technique-

All samples were collected between10.00and14.00 hours to standard is the diurnal variations of the secretion of cortisol.1 ml of the saliva were collected from patients' unstimulated glands in all 10 patients using disposable micropipettes and graduated polypropylene vials. This was followed by intra-alveolar extraction of one or more teeth under local anaesthesia. After extraction, further 1 ml of saliva was collected. Any sample with evidence of contamination by blood was discarded. Each sample was labelled and frozen until analysis (FIG 1). Saliva was analysed in ug/dl with a Salivary Cortisol ELISA research kit.

Statistical Methods:
Statistical analysis will be done by Statistical Package for the Social Sciences (SPSS) software package (SPSS 16Inc, Chicago IL, USA). The normality of data was tested by Shapiro Wilk's test. To compare the parameters between groups and within groups for normal data parametric Wilcoxon sign rank test was used among the study population. The level of significance and confidence interval was 5%and 95% respectively, i.e. $p < 0.05$. *Significant

RESULTS:
A data of total 20 samples of saliva from 15 patients before and after extraction were analysed. Out of 10 patients, 6 were males and 4 females with age ranging from 20 to 55 years with mean age of 32 years. There was a significant increase in the level of cortisol in all patients after extraction (0.83ug/dl) as compared to pre-extraction level (0.53. (Graph 1)

DISCUSSION:
Saliva maintains the homeostasis of the oral cavity through various functions such as lubrication, buffering action, maintenance of tooth integrity and anti-microbial activity. Furthermore, salivary proteins/peptides play an important role in the adherence of the oral microorganisms to the tooth surface and in maintaining the equilibrium between remineralisation and demineralization processes. The innervation and secretion of salivary glands are regulated by the ANS system, that in turn, affects salivary proteins concentration and salivary flow rate. Under repeated chronic stress conditions, the ANS system functions and consequently, the salivary glands function can get altered, which may increase risk of dental caries. On the other hand, caries-related chronic pain and dental procedures can in turn be associated with the increase of chronic stress load. Salivary cortisol level has been recognized as a valid measure of active free cortisol and as a potential stress biomarker. Many correlational studies showed a positive association of cortisol levels with chronic diseases such as periodontal diseases, diabetes, cardio-vascular diseases as well as with dental caries. Some experimental studies have shown an increase in cortisol concentrations well as in salivary total protein and secretory IgA after an exposure to experimental stress. In addition, changes in salivary composition and microbial adherence have been shown after experimental stress conditions.[7]

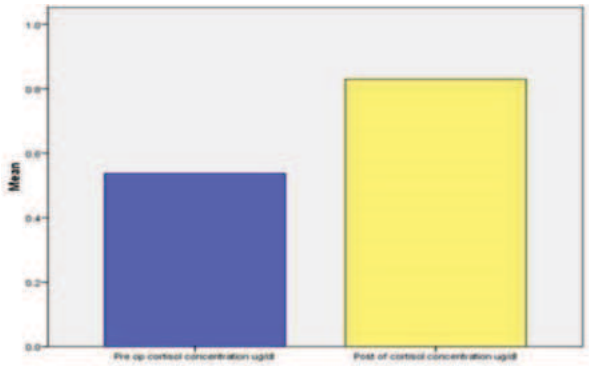
Salivary cortisol sampling has been demonstrated to be an accurate and efficient means of measuring stress response. However, the elevation elevation of salivary cortisol levels can be the result of both physiological and psychological stress.[1]

The proximity between mean cortisol concentrations before and after extraction could also be attributed to the effect of apprehension and anxiety.[3] In this present study, a significant ($p < 0.001$) difference between the concentrations before and after in patients undergoing extraction were noted. A number of studies have shown that dental anxiety and apprehension play a part in the stress response to dental treatment.[8] However, some studies have shown that some pharmacological and non-pharmacological measures are effective in that they allay patient anxiety, reduce apprehension, diminish cognitive ability, and reduce the emotional components of stress[9]. Agents such as nitrous oxide and oxygen, midazolam, and analgesics, blunt the postoperative increase in cortisol and so encourage the reduction of stress, whereas reassurance and communication of adequate information to patients about treatment, reduction of waiting time before dental procedures, sufficient time for dental appointments[10].

Relaxation, paced breathing, use of distraction techniques (such as music through headphones, video glasses, virtual reality glasses, and chewing of tasteless gum can also help to reduce stress. Some authors have also shown a close relation between salivary cortisol concentration and salivary amylase activity as indicators of stress[10]. In this study, we focused on salivary cortisol alone, however, so it may be necessary to perform further studies to compare the effect of routine intra-alveolar dental extraction on salivary amylase activity.[1]

Various literature revealed that Cortisol levels are also influenced by anaesthesia. Patients receiving treatment under local anaesthesia show lower levels of stress response then those under general anaesthesia. The efficacy of various sedatives such as midazolam, nitrous oxide, diazepam, cyclooxygenase-2 (COX-2) inhibitors or in effective melatonin have been tested using cortisol levels. The perception of pain and dental anxiety can be also altered by the use of oral contraceptives.[11] The positive correlation between the stress, anxiety and cortisol is well observed and documented in our study which revealed that salivary cortisol concentration is in its maximum level after the dental extraction procedure.[12]

CONCLUSION:
Patient's anticipation and anxiety toward dental therapy elevate the cortisol level. Dental surgeons should treat the patients more conveniently and effectively without any strains and provide a comfortable atmosphere to avoid stress-related consequences.



Graph-1: Cortisol Concentration Before And After Extraction

Figure-1: Saliva Sample Before And After Extraction

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