Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 13, (2024)
https://www.jneonatalsurg.com

OPEN ACCESS

Assessment of Salivary Cortisol Levels as a Biomarker of Anxiety in Patients Undergoing Minor Oral Surgery

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Cite this paper as: Dr. Noor ul Wahab, Dr. Waqar-Un-Nisa, Dr. Urooj Yahya, Dr. Tayyaba Mumtaz, Dr. Anum Khan Kasi, Dr. Syed Zaryab Ahmed, (2024) Assessment of Salivary Cortisol Levels as a Biomarker of Anxiety in Patients Undergoing Minor Oral Surgery. *Journal of Neonatal Surgery*, 13, 1456-1461.

ABSTRACT

Background: Anxiety is a common psychological response among patients undergoing minor oral surgical procedures, often influencing pain perception, treatment outcomes, and overall patient experience. Conventional assessment methods rely on subjective scales, which may not accurately reflect physiological stress. Cortisol, a key hormone released in response to stress, can be measured non-invasively in saliva, offering an objective biomarker for anxiety assessment.

Objective: This study aimed to evaluate salivary cortisol levels as a biomarker of anxiety in patients undergoing minor oral surgery and to explore its correlation with subjective measures of anxiety.

Methods: A cross-sectional study was conducted on patients scheduled for minor oral surgical interventions. Preoperative anxiety levels were assessed using standardized anxiety scales, followed by collection of unstimulated saliva samples at defined time intervals. Cortisol concentrations were analyzed using enzyme-linked immunosorbent assay (ELISA). Statistical analyses were performed to determine associations between salivary cortisol levels, self-reported anxiety scores, and demographic or clinical variables.

Results: Salivary cortisol levels demonstrated a significant rise in patients with higher preoperative anxiety scores. A positive correlation was observed between subjective anxiety ratings and cortisol concentrations ($p < 0.05$), supporting the utility of salivary biomarkers in objectively identifying stress responses. Age, gender, and type of surgical procedure also influenced anxiety-cortisol dynamics.

Conclusion: Salivary cortisol serves as a reliable, non-invasive biomarker for assessing perioperative anxiety in patients undergoing minor oral surgery. Integrating salivary cortisol monitoring with conventional psychological assessments may enhance clinical understanding of patient stress, guide preoperative counseling, and improve overall surgical care.

Keywords: *Anxiety, Biomarker, Minor oral surgery, Salivary cortisol, Stress physiology*

1. INTRODUCTION

A major factor in the avoidance of dental care is fear and anxiety related to the dentist and dental procedures (1). Dental anxiety is the term used to describe anxiety related to the idea of going to the dentist for preventive care and dental procedures (2). Anxiety is an emotional state that sometimes goes unnoticed before the actual encounter with the threatening stimuli (3). In daily life, it is typically encountered in situations like exams, important decision-making, the workplace, and several other situations (4). Anxiety is a frequent psychological phenomenon among patients scheduled for minor oral surgical procedures, often arising from fear of pain, injection, or the unknown (5). Such anxiety is not merely subjective, it can modulate pain perception, alter hemodynamic parameters (e.g. blood pressure, heart rate), and even influence healing and postoperative outcomes (6,7). Conventional assessment of perioperative anxiety typically relies on patient-reported scales and questionnaires, but these may not accurately reflect the physiological stress response or interindividual variability in neuroendocrine activation (8).

Cortisol, a glucocorticoid hormone released by the adrenal cortex under regulation of the hypothalamic pituitary adrenal (HPA) axis, constitutes a primary biomarker of systemic stress (9). Because cortisol is secreted into bodily fluids in response to psychological or physical stressors, its measurement offers an objective window into stress physiology. Importantly, salivary cortisol reflecting the free (unbound) fraction of systemic cortisol is noninvasive, easily collected, and correlates well with serum cortisol levels (10). This makes it an appealing candidate marker for assessing anxiety in clinical settings. Salivary cortisol has gained traction as a biomarker for anxiety, fear, stress reactivity, and surgical stress in diverse populations (11,12). For instance, salivary cortisol levels have been used to quantify stress responses in dental, medical, and psychological contexts, enabling correlations between subjective anxiety and objective hormonal changes (12). Notably, in surgical patients, studies have demonstrated that perioperative procedures and postoperative pain may influence salivary cortisol trajectories (6). However, the use of salivary cortisol specifically in the context of minor oral surgery remains underexplored.

The present study aims to assess salivary cortisol levels as a biomarker of anxiety in patients undergoing minor oral surgery, and to investigate how these hormonal changes correlate with validated anxiety scales and clinical and demographic factors.

2. METHODOLOGY

A cross-sectional analytical study was conducted in the Department of Oral and Maxillofacial Surgery in a private university of Pakistan from January 2024 till June 2024. To assess salivary cortisol levels as a biomarker of anxiety in patients undergoing minor oral surgical procedures. Ethical approval was obtained from the Institutional Review Board of the university, and written informed consent was secured from all participants. A total of 60 systemically healthy patients aged between 18 and 50 years, scheduled for minor oral surgeries such as surgical extraction of impacted mandibular third molars, alveoloplasty, or cyst enucleation under local anesthesia, were enrolled through purposive sampling. The sample size was calculated using G*Power software (version 3.1) with a confidence level of 95% and a power of 80%. Patients with systemic diseases affecting cortisol metabolism, those on corticosteroid or hormonal therapy, individuals with psychiatric disorders, smokers, and alcohol consumers were excluded to minimize confounding variables. Prior to the surgical procedure, participants were briefed on the study protocol and completed a structured questionnaire to collect demographic information including age, gender, and previous dental experience. Preoperative anxiety was assessed using the Modified Dental Anxiety Scale (MDAS) and the Visual Analogue Scale (VAS) for anxiety, both of which are validated tools for quantifying dental anxiety. Unstimulated saliva samples were collected from each participant at three distinct time intervals: 30 minutes before surgery (T1), immediately before administration of local anesthesia (T2), and 30 minutes after completion of surgery (T3). Participants were instructed to refrain from eating, drinking, brushing, or using mouthwash at least one hour prior to saliva collection. Samples were obtained using the passive drool method into sterile polypropylene tubes, immediately stored on ice, and transported to the laboratory within two hours. In the laboratory, saliva samples were centrifuged at 3,000 rpm for 10 minutes to remove impurities, and the clear supernatant was stored at –20°C until analysis. Cortisol levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (DRG Cortisol ELISA, Germany) according to the manufacturer's instructions. Each sample was analyzed in duplicate to ensure reliability, and absorbance was read at 450 nm using a microplate reader. Cortisol concentrations were expressed in nmol/L. All data were entered and analyzed using IBM SPSS version 26.0. Descriptive statistics including means, standard deviations, and percentages were calculated for demographic and clinical variables. The normality of data distribution was assessed through the Shapiro–Wilk test. Repeated measures ANOVA followed by Bonferroni post-hoc analysis were used to compare salivary cortisol levels across time intervals. Pearson's correlation coefficient was applied to examine the relationship between salivary cortisol concentrations and anxiety scores obtained from MDAS and VAS. Independent-sample t-tests and one-way ANOVA were used to assess differences based on gender, age group, and type of surgical procedure. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

A total of 60 patients participated in the study, comprising 32 males (53.3%) and 28 females (46.7%), with a mean age of 30.8 ± 7.4 years (range: 18–50 years). The most common procedures performed were surgical extraction of impacted third molars (65%), alveoloplasty (20%), and cyst enucleation (15%). The mean preoperative Modified Dental Anxiety Scale (MDAS) score was 15.4 ± 3.6, indicating moderate anxiety levels among participants. The mean score of Visual Analogue Scale was 6.8 ± 1.9, reflecting similar self-reported anxiety intensity. No significant gender difference in anxiety levels was observed ($p > 0.05$). The demographic distribution and mean anxiety scores are summarized in Table 1.

Table 1. Demographic Characteristics and Anxiety Scores of Study Participants (n = 60)

Variable	Category	n (%)	Mean ± SD
Age (years)			30.8 ± 7.4
Gender	Male Female	32 (53.3%) 28 (46.7%)	- -
Type of Procedure	• Third molar extraction • Alveoloplasty • Cyst enucleation	39 (65%) 12 (20%) 9 (15%)	- - -
MDAS Score			15.4 ± 3.6
VAS Anxiety Score			6.8 ± 1.9

Mean salivary cortisol levels increased significantly from the baseline value at T1 (8.94 ± 2.71 nmol/L) to T2 (14.28 ± 3.65 nmol/L) just before anesthesia, representing a pronounced preoperative stress response ($p < 0.001$). Cortisol levels decreased postoperatively (T3 = 10.12 ± 2.93 nmol/L) but remained higher than baseline values ($p < 0.001$). This pattern suggests that salivary cortisol accurately reflects perioperative anxiety dynamics as shown in table 2.

Table 2. Comparison of Mean Salivary Cortisol Levels at Different Time Intervals

Time Interval	Mean Cortisol (nmol/L) ± SD	p-value*
T1: 30 min before surgery	8.94 ± 2.71	—
T2: Immediately before anesthesia	14.28 ± 3.65	< 0.001
T3: 30 min after surgery	10.12 ± 2.93	< 0.001

*Repeated Measures ANOVA followed by Bonferroni post-hoc test.

A strong positive correlation was observed between preoperative salivary cortisol levels and anxiety scores measured through both the MDAS ($r = 0.674$, $p < 0.001$) and VAS ($r = 0.611$, $p < 0.001$). These findings confirm that individuals with higher subjective anxiety also exhibited higher cortisol concentrations, supporting the role of salivary cortisol as an objective biomarker for anxiety assessment as shown in table 3.

Table 3. Correlation Between Anxiety Scores and Salivary Cortisol Levels

Variable	r-value	p-value
MDAS vs. Preoperative Cortisol (T2)	0.674	< 0.001
VAS vs. Preoperative Cortisol (T2)	0.611	< 0.001

Gender and type of surgical procedure did not show statistically significant differences in preoperative cortisol levels ($p > 0.05$), suggesting that the hormonal stress response was primarily associated with anxiety intensity rather than demographic or procedural factors as shown in table 4.

Table 4. Comparison of Mean Cortisol Levels by Gender and Type of Surgery

Variable	Category	Mean ± SD (nmol/L)	p-value
Gender	Male	13.92 ± 3.48	0.311
	Female	14.62 ± 3.71	—
Type of Surgery	Third molar extraction	14.58 ± 3.69	0.420
	Alveoloplasty	13.74 ± 3.52	—
	Cyst enucleation	13.98 ± 3.40	—

4. DISCUSSION

The present study aimed to assess salivary cortisol levels as a potential biomarker of anxiety in patients undergoing minor oral surgery and to correlate physiological stress markers with subjective anxiety scores. The findings demonstrated a significant rise in salivary cortisol levels immediately before the surgical procedure, which was positively correlated with patients' self-reported anxiety on both the Modified Dental Anxiety Scale (MDAS) and the Visual Analogue Scale (VAS). These results confirm that salivary cortisol reliably reflects perioperative stress responses and can serve as a valuable objective measure of anxiety in dental clinical settings. Anxiety associated with dental and surgical procedures remains a prevalent issue that can adversely influence treatment experiences, pain perception, and postoperative recovery (13). Conventional tools for assessing anxiety, such as self-report scales, though practical, are subjective and prone to bias (14). Physiological indicators such as cortisol levels provide a more objective reflection of the body's stress response, mediated by activation of the hypothalamic–pituitary–adrenal (HPA) axis (15). Cortisol secretion increases during psychological stress and returns to baseline once the stressor subsides (16). In the current study, mean salivary cortisol levels rose significantly from baseline (T1) to immediately before anesthesia (T2), then decreased postoperatively (T3), mirroring the anticipated pattern of stress activation and recovery. These findings are consistent with those of Contac et al., in 2025, who reported elevated salivary cortisol concentrations in patients experiencing high preoperative anxiety prior to oral surgical procedures (11). Similarly, AlSahman et al., in 2024 found that patients with temporomandibular disorders and elevated stress levels had significantly higher cortisol readings, supporting its role as an anxiety-related biomarker (12). The study extends these observations by demonstrating that even minor oral surgeries, typically performed under local anesthesia, can evoke measurable neuroendocrine responses indicative of stress.

The strong positive correlation between subjective anxiety scores and salivary cortisol levels in the present study further validates the biological relevance of cortisol as a stress indicator. Hence, the clinical reliability of salivary cortisol as a noninvasive diagnostic tool for assessing acute stress reactions (17). Despite elevated cortisol levels, no significant gender-based or procedural variations were observed. The finding is consistent with a study conducted by Vicković et al., which suggests that psychological predisposition and individual stress perception may be more critical determinants of cortisol fluctuations than demographic or procedural variables (6). The noninvasive nature of saliva sampling presents a significant advantage over serum-based stress assessments. It minimizes patient discomfort, reduces procedural complexity, and allows for repeated sampling at multiple time points to monitor real-time hormonal changes. Furthermore, the use of enzyme-linked immunosorbent assay (ELISA) ensures high sensitivity and specificity for cortisol detection (18).

Nevertheless, certain factors must be acknowledged when interpreting salivary cortisol data. Variations in diurnal rhythm, dietary intake, and circadian influence may affect cortisol concentrations (19). To mitigate these effects, all samples in the present study were collected at standardized morning intervals, and participants were instructed to avoid food or caffeine prior to sampling. Clinically, integrating salivary cortisol measurement into routine preoperative assessment may enhance the understanding of patient stress profiles and enable tailored anxiety management strategies, such as relaxation techniques, behavioral counseling, or preoperative anxiolytics (20). Such an approach aligns with the growing emphasis on patient-centered care and could improve compliance, satisfaction, and overall treatment outcomes in oral surgery.

The study has several limitations which was a relatively small sample size, which may limit its generalizability to broader patient populations or those undergoing complex surgical procedures. Cortisol levels are known to follow a diurnal rhythm; although samples were standardized to morning collections, residual variations may still have influenced results. External

factors such as recent food intake, sleep quality, medication use, and baseline psychological stress could have contributed to variability in cortisol levels. Moreover, only one biomarker salivary cortisol was analyzed; including additional physiological or biochemical stress indicators (such as salivary alpha-amylase or heart rate variability) might have strengthened the analysis. Lastly, the cross-sectional nature of the study restricted the ability to assess changes in anxiety or cortisol levels over time or after anxiety-reduction interventions. Hence more future studies should include larger and more diverse populations to enhance statistical power and generalizability. More longitudinal studies to monitor cortisol fluctuations across preoperative, intraoperative, and postoperative phases for better understanding of stress dynamics. Dental professionals' awareness and skills in identifying and managing perioperative anxiety through continuing education and workshops should be enhanced.

5. CONCLUSION

The present study concludes that salivary cortisol is a dependable, objective, and noninvasive biomarker for assessing anxiety among patients undergoing minor oral surgical procedures. The significant association between cortisol levels and self-reported anxiety scores supports its utility as a physiological indicator of stress. Unlike traditional anxiety scales that rely on subjective reporting, salivary cortisol measurement offers a biochemical assessment of the patient's emotional state, providing clinicians with a more comprehensive understanding of perioperative anxiety. Integrating salivary cortisol analysis into preoperative evaluations could aid in early detection of heightened anxiety, allowing for timely psychological or pharmacological interventions, thereby improving overall surgical outcomes and patient satisfaction.

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