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Assessment of Neuroendocrine Stress Biomarkers Induced by Diverse Dental Interventions: An In Vivo Comparative Study

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ABSTRACT

Objective: Managing anxiety, fear and pain are critical components of any dental procedure. The current study quantitatively assessed the variations in salivary cortisol levels associated with stress triggered by three common dental treatments, such as teeth whitening, veneer preparation, and dental implant placement.

Aim: This *In Vivo* study aimed to assess and comparatively evaluate the variations in salivary cortisol levels associated with stress across patients undergoing teeth whitening, veneer preparation for fixed prostheses, and dental implant placement.

Methods: The levels of salivary cortisol were assessed in 80 individuals, comprising 40 healthy individuals (control) and 40 patients who underwent treatment (teeth whitening, veneer preparation, or dental implants). The patients were divided into four groups. To determine concentrations of cortisol in saliva, an Enzyme Immunoassay Kit was utilised in accordance with the manufacturer's protocol.

Saliva samples from each group underwent cortisol analysis via a validated Enzyme Immunoassay procedure calibrated specifically for salivary hormone quantification.

Results: The data revealed a stepwise reduction in salivary cortisol from Group 4 — which showed hormone levels — through Group 2 and Group 3, to Group 1, which showed minimal cortisol activity, implying that procedural invasiveness is a key determinant of the magnitude of stress in dental patients.
Conclusion: Patients who underwent implant placement showed the highest physiological stress response among all the procedures administered in the current study. Even a non-invasive procedure like teeth whitening showed a measurable elevation in the levels of cortisol, highlighting the necessity of assessing anxiety on a routine basis and management across all types of dental treatments.
Keywords: Anxiety, Cortisol level, Dental implants, Veneer preparation, Enzyme Immunoassay kit.

Introduction

Stress-related disturbances are among the most common psychological conditions encountered in healthcare [1]. Dental operatory is widely recognised as a potent catalyst of anxiety for patients undergoing any dental treatments [2,3]. Effective stress control is therefore essential to prevent loss of motivation for dental procedures. Hannibal KE and Bishop MD noted that prolonged stress responses may lead to cortisol dysregulation and widespread pain, which can further influence patient behaviour—particularly in children—and ultimately affect dental treatment outcomes [4]. Buchanan TW and Preston SD demonstrated that stress responses are not always detrimental [5]; however, reducing stress during dental procedures enhances patient adaptability and facilitates smoother treatment experiences [6,7].

Saliva can be used as a diagnostic aid for measuring stress-related biomarkers due to its ease and non-invasiveness in comparison to blood sampling. Stress-associated hormonal and enzymatic markers in saliva include alpha-amylase, secretory immunoglobulin A, cortisol, catecholamines, and chromogranin A, collectively providing a comprehensive non-invasive window for the body's stress regulatory mechanisms [8,9]. A significant difference can be seen in children's reactions to dental treatment, whether associated with painful or non-painful dental procedures. Anxiety during dental procedures can trigger stress. Furthermore, dental procedures involving local anaesthesia or discomfort are known to elevate stress levels in patients.

The dental operatory itself may act as a trigger for anxiety, including younger patients, leading to stress and anxiety [10]. Consequently, anxiety tends to be higher among individuals undergoing dental treatment, and this psychological pressure may fluctuate with successive visits depending on prior experiences.

Although a relationship between stress and pain is well established, the role of pain management in modifying this relationship remains underexplored. The present study was conducted to investigate how salivary cortisol — a well-established marker of physiological stress — responds to three commonly performed yet procedurally diverse dental treatments, specifically teeth whitening, veneer preparation, and implant placement.

Objectives

1. To establish baseline salivary cortisol concentrations in systemically healthy volunteers who received no dental intervention.
2. To determine salivary cortisol levels in patients undergoing tooth reduction and preparation for veneer as part of fixed prosthetic treatment.
3. To record fluctuations in salivary cortisol in patients receiving chairside bleaching as a non-invasive cosmetic dental procedure.
4. To quantify salivary cortisol responses in patients subjected to surgical placement of endosseous dental implants.
5. To conduct a comparative analysis of salivary cortisol-mediated stress responses across all three treatment categories and evaluate their relationship with procedural invasiveness.

Material and method

There were 80 participants aged between 20 and 30 (40 healthy individuals with no intervention and 40 patients seeking dental treatments) who enrolled on the current study. Drawing upon previously validated data reported by Gaur A et.al., the required sample size was calculated to ensure adequate statistical power of at least 80% at a 95% confidence interval, thereby ensuring the reliability of the study findings. Participants of this study were asked to give their relevant history, including medical, dental and drug history. The objectives of the investigations were explained to the participants, and they were informed of the collection of a sample of saliva. These patients underwent teeth whitening, veneer preparation for a fixed prosthesis, and dental implants.

Participants were allocated into four distinct groups based on the nature of their dental intervention:

- Group 1 (Control): Healthy individuals who received no dental treatment; only a resting saliva sample was obtained.
- Group 2: Patients who underwent tooth preparation for veneer as part of fixed prosthetic rehabilitation.
- Group 3: Patients who received in-office professional teeth whitening treatment.
- Group 4: Patients who underwent surgical placement of endosseous dental implants.

The sample of saliva for Group 1 was collected approximately five minutes before individuals were seated in the dental chair, representing a resting baseline for the study. For Groups 2, 3 and 4, the samples of saliva were gathered ten minutes after commencement of the dental procedures to capture the levels of cortisol at the peak of the procedure.

Each participant was instructed to allow natural saliva pooling and transfer the collected specimen into a sterile labelled polypropylene container. Approximately 1 mL of resting unstimulated saliva per subject was carefully preserved at a temperature of 20 degrees centigrade for laboratory investigations. Salivary cortisol concentrations were subsequently determined using the Salimetric™ Enzyme Immunoassay Kit (LLC, State College, PA, USA), following the manufacturer's recommended protocol. Data generated from the study were evaluated statistically using SPSS analytical software.

Statistical Analysis

Group-wise differences in salivary cortisol concentrations were examined through one-way ANOVA, supplemented by Tukey HSD post-hoc testing to facilitate detailed pairwise comparisons. Throughout the analysis, a p-value threshold of 0.05 was adopted as the criterion for establishing statistical significance.

Results

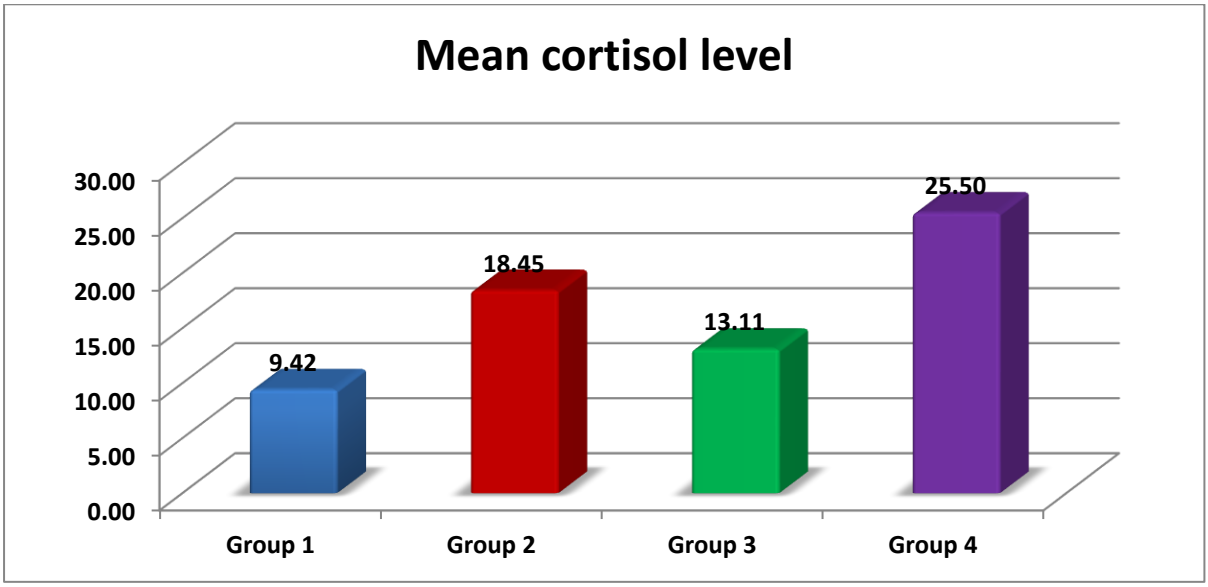
Differences in mean cortisol concentrations between the four participant groups were evaluated using one-way ANOVA. Post-hoc analysis via the Tukey HSD test was then conducted to delineate specific pairwise relationships. Across all comparisons performed, statistically significant differences were consistently identified, with p-values remaining below 0.05 in every instance. Group 4 showed highest average in levels of cortisol in saliva, significantly greater than that of Group 2. When compared directly against Group 1 controls, Group 3 participants exhibited measurably and significantly greater salivary cortisol concentrations, confirming that even non-invasive dental procedures provoke a quantifiable stress response. Group 2 values showed significantly greater than those in Group 3, reflecting a gradual decline in response to stress from surgical intervention to control conditions.

(Table 1 and Graph 1)

Groups	**Number**	**Mean**	**SD**	**F-value**	**p-value**
Group 1	40	9.42	2.98	32.242	< 0.001*[a]
Group 2	40	18.45	4.12		
Group 3	40	13.11	3.67		
Group 4	40	25.50	5.89		
Group 1 vs Group 2[b]					0.011*
Group 1 vs Group 3 [b]					< 0.001*
Group 1 vs Group 4 [b]					< 0.001*
Group 2 vs Group 3 [b]					0.039*
Group 2 vs Group 4 [b]					< 0.001*
Group 3 vs Group 4 [b]					0.011*

Table 1: Comparative mean salivary cortisol level(ng/ml) for group 1, group 2, group 3 and group 4

[a] One-way ANOVA test **[b] post-hoc Tukey test** **[c] Significant difference**



Graph 1: Comparative mean salivary cortisol level(ng/ml) for group 1, group 2, group 3 and group 4.

Discussion

The hormone cortisol is fundamentally implicated in how the brain perceives, retains, and later retrieves memories related to the stressful events, making it especially significant in understanding why dental anxiety tends to persist and intensify over time. Previous studies [11] have shown that levels of cortisol lead to the persistence of emotionally charged memories, particularly those related to fear and post-traumatic stress disorders. Lai JCL (2014) [12] further showed that variations in levels of cortisol responses to psychosocial stress are not age-dependent. Elevated and prolonged cortisol secretion demonstrates a recognised correlation with an amplified risk of depressive symptomatology and mood dysregulation, anxiety disorders, psychological distress, and reduced overall well-being.

Cortisol is secreted in response to anxiety, stress, and physical or psychological pressure. Levels of cortisol may also elevate during early morning awakening, physical exertion, or acute emotional tension. Physiologically, cortisol supports the "fight-or-flight" response by increasing glucose availability to major muscle groups. It simultaneously inhibits insulin release to prevent glucose storage, ensuring rapid energy accessibility when required [13].

The present study evaluated salivary cortisol responses to stress induced during teeth whitening, veneer preparation for fixed prosthesis, and dental implant procedures. A significant difference in the levels of cortisol was observed in individuals undergoing treatment (Groups 2, 3, and 4). The highest cortisol levels were recorded in Group 4 (patients undergoing dental implant placement), followed by Group 2 (veneer preparation), Group 3 (teeth whitening), and the lowest levels in Group 1 (control). These findings align with previous studies by Padmanabhan V, Rai K, Hedge AM[10] and Phukela SS et al.[14], which demonstrated that dental procedures consistently elevate salivary cortisol levels compared with non-treated controls.

Exposure to dental procedures heightened stress and anxiety, resulting in elevated salivary cortisol levels among Groups 2–4. Similar observations have been documented in studies by Phukela SS et al. [15,16]. Among the treatment groups, implant placement (Group 4) elicited the highest cortisol levels, likely due to the procedural invasiveness itself and the anticipatory anxiety surrounding local anaesthetic administration, both recognised as independent contributors to elevated psychological distress in dental patients.

Cortisol levels in Group 2 (veneer preparation) were higher than in Group 3 (teeth whitening), potentially due to the greater invasiveness of tooth preparation and the associated sound, vibrations, or minor bleeding. Group 3 also exhibited increased cortisol levels compared with controls, indicating that even minimally invasive cosmetic procedures can induce stress and anxiety in dental settings.

Conclusion

1. The stress level was highest when patients underwent dental implants, followed by Group 2 (veneer preparation for fixed prosthesis) and Group 3 (teeth whitening).
2. Healthy controls with no intervention showed the lowest cortisol concentrations, establishing a clear baseline reference for comparison between procedural stress.
3. The level of cortisol in saliva can be seen as one of the reliable indicators for the detection of stress.

Strengths

Employing salivary cortisol as the primary outcome measure offered a significant methodological advantage: unlike serum cortisol, which is mainly attached to corticosteroid-binding globulin, salivary cortisol indicates the biologically active, unbound portion of the hormone. This distinction ensures that salivary evaluations more accurately reflect true HPA axis activity, free from the confusing influences of binding proteins present in blood.

Limitations

The levels of cortisol in saliva were not assessed at a specific time. Therefore, diurnal variations may have occurred at some degree of baseline variability.

Future Directions

Future research should explore:

1. Evidence-based strategies for reducing pre- and intra-procedural dental anxiety include cognitive behavioural approaches, guided relaxation techniques, pre-appointment counselling, and pharmacological anxiolysis, as well as their impact on salivary cortisol levels.
2. The long-term systemic implications of repeated elevation in cortisol levels.

Conflict of Interest

None of the contributing authors declared any financial or personal conflict of interest pertaining to the research presented in this article.

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