



# Assessment of Salivary Cortisol Level to Evaluate Stress among Dental Undergraduates- An In vivo Observational Study.

(Between Books and Burs: Assessing Stress in Dental Undergraduates via Salivary Cortisol)

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## DECLARATION OF PATIENT CONSENT

The authors certify that they have obtained all appropriate patient consent forms. In the form, the participants have given their consent for their clinical information to be reported in the journal. The participants understand that their name and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Nil.

## CONFLICTS OF INTEREST

There are no conflicts of interest.

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KEYWORDS	ABSTRACT:
Salivary cortisol, dental students, stress, non-invasive biomarker, dental education, clinical performance	<p><b>Background:</b> Dental education is often associated with high levels of psychological stress, which may adversely affect students academic performance and clinical efficiency. Salivary cortisol is a non-invasive biomarker that reflects physiological stress responses.</p> <p><b>Aim:</b> To evaluate and compare salivary cortisol levels between two groups of dental students and assess the extent of stress experienced.</p> <p><b>Materials and Methods:</b> A cross-sectional study was conducted among 80 dental students, divided equally into Group A and Group B. Unstimulated saliva samples were collected and analyzed for cortisol concentration using standard immunoassay techniques. The data were statistically analyzed using an independent t-test.</p> <p><b>Results:</b> Group B exhibited significantly higher salivary cortisol levels (Mean = 0.202 ± 0.046) compared to Group A (Mean = 0.174 ± 0.042), with a p-value of 0.005, indicating a statistically significant difference (p &lt; 0.05).</p> <p><b>Conclusion:</b> Dental students in Group B demonstrated elevated salivary cortisol levels, suggesting increased physiological stress. These findings emphasize the need for integrating stress assessment and management strategies into the dental curriculum to support student well-being and optimize clinical performance.</p>



## INTRODUCTION:

Dental curriculum is designed to train a dental student to expertise in clinical, academic and research. This enables them to get trained and serve the patient in most efficient way for years. Through the undergraduate program the dental students are put under tremendous physical and mental work to meet up their academic curriculum includes heavy workload, managing uncooperative patients ,high parental expectations and life style. Previous studies is evident to show that dental students have higher level of stress than the general population. Increase in stress causes the body to release cortisol in increased level.

Cortisol causes several systemic conditions like increases gluconeogenesis, suppress insulin secretion, anti-inflammatory function, diurnal variation, immunosuppression. Upto 95% of the secreted cortisol is bound to large proteins [corticosteroid binding globulin (CBG) albumin] and transported in blood with small fraction of unbound free cortisol that is biologically active. These conditions are reflected on the saliva. Salivary cortisol is frequently used as a biomarker for assessing and correlating to psychological stress. The favourable attributes of saliva over other biofluids are non invasiveness ,self collection, self handling, economy and easy storage. Microbial and molecular elements in saliva have led to the development of ‘Salivaomics’ emphasising the role of saliva to the entire physiological system. The first two years of dental curriculum was dealt to deal with basic sciences and preclinical works where the students will not be exposed to any clinical program. <sup>1,2</sup>

The transition from the preclinical to clinical that happen in the third year of the dental program where the students are exposed to clinical environment. This might increases the chances of high stress during the third year. The assessment of cortisol level during such transition period might enable us to effectively correlate with the significant stress level among the dental students. The data concerning such clinical assessment was not sufficient. The aim of the present study was to compare the stress levels among the third year undergraduates dental students, and evaluate the role of salivary cortisol as a stress biomarker. <sup>2</sup>

## MATERIALS AND METHODS:

**ARAMENTARIUM:** 1. Precision pipettes 2. Disposable pipette tips. 3. Distilled water 4. Plate shaker 5. Benchtop centrifuge 6. Microplate reader 7. Calibrator

**INCLUSION CRITERIA:** Students above 18 years of age and without any systemic illness were included in the study.

**EXCLUSION CRITERIA:** Students with a drug history of corticosteroids, anti-depressants, were excluded from the study. Students experiencing pre-menstrual symptoms, pregnancy and familial issues.

**METHODOLOGY:** The study was approved by the Institution Research Board. This study was conducted among the third-year undergraduate students. Informed consent was obtained from voluntary participants of the study.

A total of 40 participants were included in the study. Students above 18 years of age and without any systemic illness were included in the study. Those students with a drug history of corticosteroids, anti-depressants, were excluded from the study. Female students experiencing pre-menstrual symptoms, pregnancy, and familial issues were excluded from the study. To measure the physiological levels of stress, two salivary samples were obtained from each subject : The first sample was collected before entering the clinical postings in the morning and the second sample was collected in the evening after leaving the classroom.

The participants were instructed to refrain from intake of acidic drinks, milk and solid foods for at least 1 hour before the collection of sample. The participants were trained on the method of saliva collection. The participants were asked to sit in a erect position and asked to accumulate saliva for 5minutes. Approximately 4 to 5ml of unstimulated saliva was collected on a container. Saliva was collected by a spitting method into a collection device and stored at -20°C until processing.

The collected sample was immediately stored at 4°C until processing. Salivary cortisol levels were assessed by ELISA method using CORTISOL SALIVA ELISA – Diagnostics Biochem Canada Inc. The data were analysed using statistical package. Paired sample t-test was used to compare the diurnal variation between morning and evening samples within the students.



### STATISTICAL ANALYSIS:

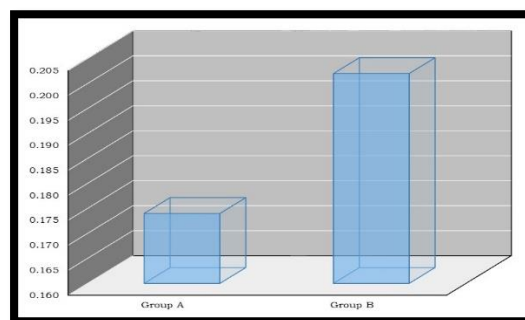
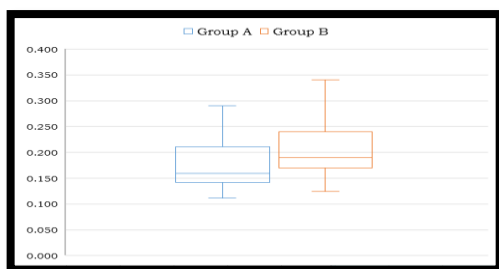
The data were entered in Microsoft Excel and analysed statistically using the SPSS software, version 21; SPSS Inc., (Chicago, IL, USA). The normality of the data was assessed prior to analysis using the Shapiro-Wilk's test/Kolmogorov-Smirnov test. Data were found to be normally distributed. Thus, parametric test was chosen. Descriptive statistics were used to calculate frequencies, percentages, and mean values. Paired t test and unpaired t test were carried out to determine the difference between the groups. All statistical tests were performed at a significance level of 5% ( $p < 0.05$ ).

### RESULTS:

**TABLE 1: INTERGROUP COMPARISON**

	N	Mean	Standard Deviation	p-value
Group A	40	0.174	0.042	0.005*
Group B	40	0.202	0.046	
p-value based on Independent-t-Test				
* = Statistically Significant ( $p < 0.05$ )				

An independent t-test was performed to compare the mean salivary cortisol levels between Group A and Group B. The results indicated that Group B (Mean = 0.202, SD = 0.046) had significantly higher cortisol levels compared to Group A (Mean = 0.174, SD = 0.042). The difference was statistically significant with a p-value of 0.005 ( $p < 0.05$ ). This suggests that Group B experienced a significantly greater physiological stress response than Group A, as reflected by elevated salivary cortisol concentrations.



### DISCUSSION:

Dental students experience several challenging problems on their daily routine. According to the literature, dental students are prone to stress. Stress has a huge impact in students mental health and affects the overall academic performance. The stress response has been initiated by anxiety, which is the psychophysiological signal. A significant workload to finish within a short time makes the clinical students more anxious. Students who reside in hostels are more likely to experience stress than who live with their families. Stress that is not controlled would lead to increased smoking, substance abuse, insomnia and eating disorders.<sup>3,4</sup>

A study by Ghodrat et al., 2014 has revealed the effect of stress on the process of memory. Stress is capable of causing functional and structural changes in the hippocampus area of the brain, as evidenced by various studies (McEwen,1999. Lupien and Lepage,2001). Cognition can be affected by stress in different ways, depending on its intensity, duration, origin and magnitude. Counselling can be arranged for students to prepare them mentally before entering the clinicals. Regular workshops can be organized to promote emotional intelligence, teach time management skills, coping with patients who are not cooperative and motivate students to handle stress in college life.<sup>5,6</sup>

The ability of students to handle stress is crucial and may aid them in their future careers. The importance of sleep, diet, exercise and stress-relieving activities like yoga and medication should be discussed to enhance overall wellness among the dental students.<sup>7</sup>

The present intergroup comparison revealed a statistically significant difference in salivary cortisol levels between the two groups. Group B demonstrated



higher mean cortisol levels compared to Group A, indicating a greater physiological stress response. These findings suggest that Group B was exposed to or perceived higher stress, as evidenced by elevated salivary cortisol concentrations. Salivary cortisol may thus serve as a reliable, non-invasive biomarker for assessing stress levels in similar population settings.<sup>8</sup>

## CONCLUSION:

The present study revealed a statistically significant increase in salivary cortisol levels among dental students in Group B, indicating a higher physiological stress response compared to Group A. These results highlight the importance of recognizing stress as a critical factor in dental education, with potential implications for academic performance and clinical competency. Salivary cortisol serves as a practical, non-invasive biomarker for early identification of stress among students. Integrating stress management strategies and mental health support within the dental curriculum is essential to promote student well-being and enhance clinical outcomes.

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