ORIGINAL ARTICLE

Salivary Cortisol as a Stress Biomarker in Fourth-Year Dental Students: from First Patient Touchpoint to Clinical Immersion

Hind Hadi Enad[1,2], Mustafa Wathiq Adbul Kareem[2], and Mohammed Abbood Al-Maliky[2]

[1] Department of Dental Science, Advanced Medical and Dental Institute, Universiti Sains Malaysia,13200 Kepala Batas Pinang, Malaysia
[2] Department of Dentistry, Al-Hadi University College, 10011 Baghdad, Iraq

ABSTRACT

Background: Stress is prevalent among dental students due to rigorous demands. This study evaluates salivary cortisol as a stress biomarker in fourth-year dental students at Al-Hadi University College, Iraq. **Materials and Methods:** A longitudinal design involved random sampling of 52 fourth-year dental students. Ethical approval was obtained prior to the study. Unstimulated saliva (3 mL) was collected at the start and after two months of clinical exposure. Samples were centrifuged, and cortisol levels were analyzed using the Elecsys Cortisol II kit on the Cobas e411 system. Inclusion criteria were students aged 22 to 26 years with no medical conditions. Exclusion criteria included irregular attendance, smoking, and pregnancy/lactation. Data were analyzed using SPSS version 26. **Results:** Initial stress levels averaged 16.09 nmol/L, decreasing to 14.48 nmol/L after two months. Salivary cortisol levels significantly reduced (t-value = 3.02, p-value = 0.004). **Conclusion:** Fourth-year dental students showed reduced stress levels and salivary cortisol over time, indicating adaptation to the clinical environment. The two-month period effectively observed physiological changes. Transitioning to real patients improved performance through reduced procedure times and higher case marks. Repeated clinical exposure enhances coping mechanisms and performance.

Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP10):104-109. doi:10.47836/mjmhs.21.s10.21

Keywords: Dental students; restorative; salivary cortisol; stress; biomarker

Corresponding Author:
Hind Hadi Enad, MSc
Email: Hind87@student.usm.my
Tel :+9647819950619

INTRODUCTION

Stress, as conceptualized by Hans Selye in the 1930s, is a fundamental component of our everyday lives. It involves an active interaction between an individual's abilities and the demands of a given situation. While it can somewhat enhance performance, prolonged stress can have adverse physical and mental effects (1). The British Association for Counselling and Psychotherapy has reported a significant increase in the number of students experiencing psychological disorders due to stress in recent years (2). Dental students are expected to fulfil more than just theoretical requirements, it might be argued that they are more susceptible to protracted stress than students in other academic areas; this career emphasizes the importance of clinical practical skills and establishing solid connections with patients (3).

Throughout their education, students are subjected to various stressors, such as severe workloads, timetable pressure, and the need to balance acquiring fundamental information with perfecting specialized clinical skills. In the hands-on phase, students face various pressures, including patient interactions, care responsibilities, and challenges in acquiring manual skills (4). Unmanaged stress can result in negative consequences such as academic underachievement and psychological disorders. Environmental, economic, and cultural factors can influence students' perceived stress levels by decreasing, increasing, or altering them (5).

Intensive engagement between the dentist and the patient may result in 'burnout,' which includes emotional exhaustion. Stress among dental students has emerged as a critical problem for dental educators in recent decades (6).

Numerous stress management strategies exist focusing

on promptly identifying stress-induced harm through biomarkers. Limited attempts have been made to quantify stress levels through a biomarker. Acute stress activates the hypothalamic-pituitary-adrenal (HPA) axis, leading to elevated cortisol levels. Researchers have demonstrated that salivary cortisol is a reliable biomarker for measuring acute stress in various stressful scenarios (1).

In response to stress, the adrenal cortex releases cortisol, a hormone that is well-known as the stress hormone. Saliva cortisol concentration measurements have several advantages over the more common total serum measurements. Saliva collection for cortisol measurement is simple, non-invasive, and unlikely to trigger a stress response, unlike blood sampling, which requires venipuncture and may itself elevate cortisol levels (7,8,9). Cortisol levels in saliva are proportional to the proportion of plasma and serum cortisol that is physiologically active, unbound, and unrelated to the saliva flow rate (10, 11).

Blood cortisol concentrations peak approximately 15–30 minutes after a stressful event, and progressively decline until they return to pre-stress levels 60–90 minutes later. The high diffusivity of cortisol into saliva ensures that blood and saliva levels are constantly linked. Given the hormone's diurnal variation, cortisol levels peak shortly after awakening and gradually decline throughout the morning. To ensure consistency and minimize variability, saliva samples are commonly collected between 8:00 AM and 11:00 AM, when cortisol is still relatively high but past its peak, maintaining measurement stability (12). As a result, salivary cortisol was chosen over blood cortisol due to its non-invasive, stress-free collection method, making it particularly suitable for repeated measurements in a clinical setting. Unlike blood sampling, which requires venipuncture and may itself trigger a stress response, saliva collection is painless, simple, and does not require specialized personnel, allowing for more natural and reliable stress hormone measurements (13,14).

Psychological stress among dental students is well recognized, but most research still relies on self-reported questionnaires. Salivary cortisol, a well-established biomarker of stress, provides a non-invasive, objective measure of physiological responses. Clinical exposure marks a demanding transition as students move from simulation to real patient care, facing emotional, technical, and communication challenges.

This study explores stress through salivary cortisol levels in fourth-year dental students during their initial clinical exposure, aiming to track changes over two months and assess whether short-term physiological adaptation occurs. The findings offer important insights into how stress changes during this critical phase, supporting strategies to promote student well-being and strengthen clinical training.

MATERIALS AND METHODS

Study Design

This study employed a longitudinal design to investigate the changes in salivary cortisol levels among fourth-year dentistry students during their initial exposure to the restorative clinic and after two months of practical experience.

Study Participants

A total of 95 fourth-year dental students from Al-Hadi University College were initially considered for participation. After applying the inclusion and exclusion criteria, 73 students met the eligibility requirements. From this group, 52 students aged 22 to 26 were randomly selected using Excel-based randomization, ensuring an unbiased selection process.

A sample size calculation was performed using G*Power version 3.1.9.7 (Universitɑt Dьsseldorf, Germany). Based on a medium effect size (Cohen's d = 0.5), an alpha level of 0.05, and a power of 0.90 for a two-tailed paired t-test, a minimum of 44 participants was required. Considering an anticipated dropout rate of 20%, a target sample size of 53 participants was determined. Ultimately, 52 students volunteered and were included in the study.

The group included both males (n=24) and females (n=28). Prior to the study, ethical approval was obtained. Students with any medical conditions or medications known to affect cortisol levels were excluded, as well as those who were smokers, pregnant women, or breastfeeding mothers. Additionally, students who experienced significant external stressors during the study period, were excluded to minimize the impact of external stress on cortisol levels. Repeater students with prior clinical experience were also excluded to ensure that all participants were undergoing their first clinical exposure, maintaining consistency in stress adaptation patterns.

Saliva Sample Collection and Processing

A total of 3 mL of unstimulated saliva was collected from each participant over a 10-minute period while they were seated in a relaxed, slightly forward-leaning position to allow passive drooling into sterile containers. Collection was performed in the morning between 8:00 AM and 11:00 AM to minimize the effects of cortisol's diurnal variation. Participants were instructed to refrain from eating for at least two hours prior to collection. Saliva was collected at two intervals: once at the beginning of the academic year (early October) and again two months later (mid-December). Immediately after collection,

samples were placed in an ice container and transported to the laboratory. The saliva samples were centrifuged at 3000 rpm for 15 minutes to separate debris, and the clear supernatant was transferred into microcentrifuge tubes and stored at −20°C until biochemical analysis.

Prior to analysis, the frozen samples were brought to room temperature.

The Elecsys Cortisol II immunoassay (Roche Diagnostics, Germany) was used on the Cobas e411 fully automated system (Roche Diagnostics, Germany). The test is based on a competitive electrochemiluminescence immunoassay principle, where cortisol in the sample competes with a ruthenium-labeled cortisol derivative for antibody binding. After incubation with streptavidin-coated microparticles, the immune complex was magnetically captured, and a chemiluminescent signal was measured. Cortisol concentration was determined using a two-point calibration curve, with results expressed in nmol/L.

Statistical analysis

Descriptive analysis was conducted for all variables. Each student provided two saliva samples, one at baseline (T0) and another after two months (T1), resulting in a total of 104 measurements. Normality of the differences between paired salivary cortisol measurements was assessed using the Shapiro-Wilk test, and the data were found to be normally distributed ($p = 0.548$). Therefore, paired t-tests were used for comparisons, with statistical significance set at $p \leq 0.05$. Data were analyzed using SPSS version 26, with results presented as mean ± Standard Deviation (SD).

Ethical clearance

This study was approved by Research Ethics Committee, Department of Dentistry, Al-Hadi University college No. HD122311/2023

RESULTS

This study examines stress and salivary cortisol levels among dental students, comparing their initial experiences at the restorative clinic to observations after two months of clinical engagement.

A total of 52 fourth-year dental students participated, with an age range of 22 to 26 years. The majority of participants were 22 years old (n = 27, 52%), with a nearly equal distribution of females (n = 28) and males (n = 24) as shown in Table I.

Salivary cortisol levels were assessed at two time points: before clinical exposure and after two months of training. Upon their introduction to the restorative clinic, participants had an initial mean cortisol level of 16.09 nmol/L (SD = 3.16). Remarkably, subsequent measurements conducted after two months demonstrated a discernible reduction, with cortisol levels decreasing to 14.48 nmol/L (SD = 4.15) (Table II). This decline suggests a potential familiarization with the clinical setting and a physiological adjustment to the demands of patient care.

Table I: Demographic Characteristics of Participants

Age (years)	Female (n)	Male (n)	Total (n)
21	1	1	2
22	16	11	27
23	5	2	7
24	2	4	6
25	3	4	7
26	1	2	3
Total	28	24	52

Table II: Salivary Cortisol Levels (nmol/L) at Two Time Points

Variable	t-value*	Significance (Sig.)	Mean (Standard Deviation, SD)	
			Mean (SD) (T0)	Mean (SD) (T1)
Salivary Cortisol Levels (nmol/L)	3.02	0.004	16.09 (3.16)	14.48 (4.15)

*Paired samples t-test

The initial cortisol measurements served as a foundational reference point, allowing for a direct comparison after two months of exposure to clinical practice. Statistical analysis revealed a significant difference between the two time points (t-value = 3.02, $p = 0.004$), confirming a measurable reduction in cortisol concentrations over time.

DISCUSSION

Examining the pressures dental students face and their self-assurance in carrying out various procedures is crucial for educators to consistently assess and enhance clinical instruction, guaranteeing the graduation of proficient practitioners.

Our study observed a progressive decline in salivary cortisol levels among 4th-year dental students at Al-Hadi University College from their first clinic session to after eight clinic sessions, indicating a consistent reduction in stress levels as students become more accustomed to the clinical environment. The normal morning (8:00 AM) salivary cortisol range is 0.69–16.6 nmol/L (15). The students' initial cortisol levels (16.09 nmol/L) were near the upper limit of this range, indicating a high physiological stress response at the beginning of clinical training. The hypothalamic-pituitary-adrenal (HPA) axis plays a central role in mediating the physiological response to stress, leading to cortisol secretion (16). In the present study, salivary cortisol levels were elevated at the start of clinical exposure (mean = 16.09 nmol/L) and declined significantly after two months (mean = 14.48 nmol/L). This pattern reflects the initial acute activation of the HPA axis during early clinical training, followed

by regulation and adaptation over time as students become more familiar with clinical procedures and patient interactions (17).Despite still being within the normal range, this decline highlights the body's ability to regulate stress responses over time as students gain confidence and familiarity with clinical procedures.

The observed drop in cortisol levels may be attributed to several factors. As students become more familiar with clinical procedures, their initial anxiety and stress likely diminish. Furthermore, repeated exposure to clinical settings may enhance their coping mechanisms and confidence in handling patient interactions. Given these findings, the two-month period in our study was appropriate for assessing early stress adaptation in fourth year dental students.

Previous studies frequently compare stress levels between dental students and those in other healthcare fields. Ersan et al. (18) observed that dental students experience higher stress than medical students, a finding initially reported by Murphy et al. (19). This heightened stress level can be attributed to several factors unique to dental education. Unlike medical students, who often engage in team-based learning and supervised clinical observations, dental students are required to perform hands-on procedures independently at an early stage of their training. The pressure of working on real patients, fear of making irreversible mistakes, and the need to meet strict clinical requirements contribute significantly to increased stress levels. Moreover, dental curricula are often structured with intense workloads, frequent practical assessments, and limited flexibility, leading to higher anxiety and emotional exhaustion (20). In contrast, our research focuses exclusively on dental students, providing a more in-depth look at their stress levels over time.

To better understand how stress dynamics during clinical training vary globally, comparisons with findings from other countries were explored.

Several studies have reported an increase in stress levels during the clinical phases of dental education, consistent with the present findings. Studies from Greece (4) and Australia (21) observed heightened stress as students transitioned into clinical practice. Early clinical exposure, with its demands for real-time decision-making and fear of patient-related errors, has been identified as a key contributor to elevated stress levels among dental students (22). These findings suggest that direct patient care, academic pressures, and clinical skill acquisition contribute to elevated stress during this stage.

In contrast, some studies did not observe a consistent trend of increasing stress levels. Research from the United States (23) found no significant difference in stress across academic years, while a study from Saudi Arabia (24) reported no clear correlation between stress and clinical workload. These discrepancies may reflect differences in study design, educational systems, cultural expectations, or stress management resources across countries.

Additionally, some studies have reported no clear correlation between stress levels and academic performance among university students (24, 25), suggesting that stress does not uniformly predict academic outcomes across different educational contexts.

In the present study, students exhibited elevated salivary cortisol levels at the start of clinical exposure, reflecting acute physiological stress. However, a significant decline in cortisol was observed after two months, indicating physiological adaptation. Similar patterns have been reported in recent studies. Propp et al. (26) found a reduction in cortisol awakening response in medical students during repeated acute surgical simulations. Rɯsner et al. (27) demonstrated that individuals with higher resilience exhibited a greater decline in cortisol outputs after repeated social stress. Batabyal et al. (28) also observed a decline in salivary cortisol among male undergraduate students over an academic year. These findings, together with our results, suggest that repeated exposure to clinical environments facilitates HPA axis regulation and reduces physiological stress over time.

This study's primary limitation lies in its reliance solely on salivary cortisol measurements to assess stress adaptation during initial clinical exposure. While it provides valuable insights, future studies could benefit from comparing self-reported stress levels with biochemical data. Comparing results across different universities or student groups could also add valuable context. Although conducted in a single setting, this study provides a strong basis for future research into stress and adaptation in clinical education.

CONCLUSION

This study sheds light on how dental students biologically adapt to the stresses of early clinical training. The steady decline in salivary cortisol levels suggests that while the first exposure to patient care can be highly stressful, students gradually develop resilience as they gain experience and confidence. These findings highlight that early clinical stress is not only normal but also manageable with the right support. By introducing mentorship programs, stress management training, and gradual clinical exposure, dental schools can help students navigate this critical phase more smoothly, protecting their well-being and strengthening their readiness for professional practice.

ACKNOWLEDGEMENTS

We express our gratitude to everyone who provided

professional assistance during this research, including colleagues and participants, for their invaluable support.

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