RESEARCH ARTICLE DOI: 10.53555/vmrx7870

# IMPACT OF ANATOMICAL DISSECTION EXPOSURE ON SALIVARY CORTISOL AND PERCEIVED STRESS IN MEDICAL AND DENTAL STUDENTS

Suha Mahrukh <sup>1</sup>, Amna Akram <sup>2</sup>, Awais Anwar <sup>3\*</sup>, Hasan Najeeb Khan <sup>4</sup>, Lubna Yousaf <sup>5</sup>, Naveed Shuja <sup>6</sup>

<sup>1</sup>Lecturer of Anatomy at Muhammad Medical College, Ibn E Sina University Mirpurkhas

<sup>2</sup>Assistant professor department of Biochemistry Sahara Medical College Narowal

<sup>3\*</sup>Professor of physiology. Sahara Medical College Narowal.

<sup>4</sup>Rawal Institute of Health Sciences, Islamabad

<sup>5</sup>Assistant Professor Community Dentistry Avicenna Dental College Lahore

<sup>6</sup>Professor department of Biochemistry Lahore Medical and Dental College Lahore

\*Corresponding Author: Dr. Awais

\*E-mail: <u>Anwarawaisanwar157@gmail.com</u>. contact no.923364483322

#### Abstract

**Background:** Cadaveric dissection remains a core aspect of medical and dental education, offering the invaluable three-dimensional anatomy. However, one can experience severe physiological and psychological stress when first subjected to preserved cadavers. This experiment had compared the changes in salivary cortisol and the perceived stress levels among the MBBS and BDS students during the first session of gross anatomy dissection study.

**Methods:** A quasi-experimental observational study was conducted among 100 first year students (50 MBBS, and 50 BDS) of a teaching institution. Salivary cortisol and Perceived Stress Scale (PSS-10) scores were measured at two levels and these were measured at 15 minutes during entrance of the dissection hall (T1) and 15 minutes during completion of 90 minutes of a normal cadaveric dissection procedure (T2). Samples processing involved use of a high-sensitivity ELISA kit and PSS-10 scores were used to measure subjective stress. Paired t-tests, Wilcoxon test and correlation were used to analyze.

**Results**: MBBS and BDS students had a noticeable increase in salivary cortisol after dissection (MBBS:  $9.82 \pm 2.15$  to  $14.26 \pm 2.87$  ng/mL; BDS:  $10.11 \pm 2.31$  to  $15.02 \pm 3.01$  ng/mL; p < 0.001 in both cases). Perceived stress also rose (MBBS:  $15.4 \pm 4.2$  to  $15.4 \pm 4.9$ ; BDS:  $16.1 \pm 4.6$  to  $16.1 \pm 4.6$  to

**Key words:** Cadaveric dissection; Salivary cortisol; Perceived stress; Medical students; Dental students; HPA axis; Anatomy education;

#### Introduction

Since ancient times, anatomical dissection has been considered a rite of passage in medical and dental educational history, as an essential practice in development of spatial knowledge, diagnosis, and professional embodiment <sup>1</sup>. Although digital simulation, virtual anatomy and high-resolution image systems have advanced rapidly, the cadaveric dissections have not been able to match the ability of the cadaveric system to provide the feel of the human structure in three dimensions <sup>2</sup>. The experience of getting exposure to the dissection hall, however, is one of the first experiences of medical trainees to death, biological decay, and the intensity of sensations of preserved human tissue. Such an experience is not only pedagogically reflective but also psychologically challenging <sup>3</sup>. To most students, the initial experience with cadaver will induce a certain level of curiosity, respect, discomfort, and anxiety, which triggers a state of stress that may impact physiological processes, emotional control, and educational results <sup>4</sup>.

The dissection stress is not just a subjective one. Exposure to preserved cadavers under controlled conditions has been reported to stimulate Hypothalamic-pituitary-adrenal (HPA) axis leading to quantifiable rise in the amount of circulating cortisol, the main endocrine mediator of acute stress <sup>5</sup>. Salivary cortisol is a more accurate, non-invasive biomarker of HPA activity, which is sensitive to a dynamic change in physiological arousal and is extensively utilized in the study of stress reactivity in behavioral neuroscience, psychophysiology, and medical education studies. Higher levels of cortisol can temporarily positively affect alertness and encoding of memory, but prolonged or excessive stimulation may interfere with the efficiency of learning, emotional state, and resilience <sup>6</sup>. With knowledge on how anatomical dissection can alter cortisol secretion, thus, gives pivotal information on how students will physiologically adjust to this distinctive learning setting <sup>7</sup>.

Simultaneously with neuroendocrine assessments, perceived stress is an evaluation of a psychological interpretation of the dissection experience. Anxiety, sleep disturbance, nausea, intrusive images and tension of emotion are often reported by students before or during early dissection sessions. Although the majority can adapt with repeated exposure, a sub-group can display sustained distress, which has consequences of burnout, development of professional identity and mental-health susceptibility <sup>8</sup>. Students of medicine and dentistry may have different patterns of physiological and psychological reactivity, due to their different training structures, their intensity of the curriculum, and their exposure to cadavers. However, there is a dearth of direct comparative evidence available especially in lower- and middle-income nations where the social-cultural perceptions of death, body donation, and handling of cadavers may also influence stress reactions <sup>9</sup>.

With the medical education field developing globally toward focusing on well-being, resilience, and humane pedagogy, it becomes necessary to learn about the biological and psychological effects of early curricular stressors. Although the objective biomarkers of dissection exposure like salivary cortisol and subjective measures of the same are treated with increasing awareness, the literature has not done enough to integrate the two <sup>10</sup>. Such knowledge gap limits the creation of evidence-based interventions, including preparatory workshops, guided orientation, reflective practices, peer mentoring, and better cadaver preparation standards, which might reduce stress and still have an educational rigor. The study examines the effects of exposure to anatomical dissection on salivary cortisol and perceived stress levels in medical and dental students and offers a comprehensive analysis of the effect early in the professional career on stress reactivity <sup>2,7</sup>. The proposed study will clarify the inter-program variations, define the vulnerable groups of people, and offer a better understanding of anatomy education through a more humane and scientifically oriented approach by integrating the endocrine indicators with the validated psychological outcomes.

### **Materials and Methods**

The study was designed as an observational, quasi-experimental investigation conducted at Sahara Medical College Narowal and Rawal Institute of Health Sciences Lahore. It aimed to evaluate the acute physiological and psychological stress responses associated with first-time exposure to the cadaveric dissection hall. The data were gathered throughout a four weeks period as the first gross anatomy dissection classes of the academic year were proceeding. One hundred first year students

(50 BDS and 50 MBBS) were recruited using consecutive sampling. Only students attending the first year of professional studies, who answered on the day of planned dissection, and signed written consent to salivary sampling and psychological tests were involved. Students with history of known corticosteroid or psychotropic medication use, acute illness, chronic endocrine disorders, psychiatric diagnosis and exposure to major stressful events in the last 72 hours were excluded. The sample was also not eligible of individuals who had consumed caffeine or nicotine, or performed hard physical activities in the last two hours.

Ethical permission on the research was taken out with the Institutional Review Board / Ethical Review Committee of the host institution and there was strict confidentiality and anonymity. All the participants were provided with a standardized orientation session before the sampling process where the aim of the study, saliva collection methods, and precautions taken to minimize the possible confounders such as not eating, drinking, brushing teeth, or smoking at least 30 minutes prior to the collection process were elaborated. This was followed by exposure of the students to their introductory cadaveric dissection session, under the controlled environmental conditions with fixed light and temperature, and exposure to formalin. Every session was 90 minutes; it comprised cadaver unveiling, orientation, and supervised dissection.

Salivary cortisol was measured at two points T1, 15 minutes prior to entering the dissection hall and T2, 15 minutes following the 90 minutes dissection session. The samples of saliva were taken with sterile salivate cotton swabs (Sarstedt, Germany), which remained in the mouth during 90 seconds. The samples collected were coded, kept on ice and then taken to the lab promptly and centrifuged at 3000 rpm and a period of 10 minutes. The obtained supernatant was aliquoted into sterile cryovials, and the amount of cortisol at high sensitivity level was measured using high sensitivity ELISA kit that had been validated on salivary biomarkers. All samples were handled in less than two hours and all assays were done in duplicates in order to have precision. Six standards (0-40 ng/mL) were used to draw-up the calibration curves and the laboratory technicians were blinded to the identity of the participants in order to reduce bias.

To measure psychological stress, we used the 10- Item Perceived Stress Scale (PSS-10), which is a validated instrument to measure subjective stress. The questionnaire was filled at the two time points as the collection of cortisol was carried out to coincide in the evaluation of physiological and psychological. The answers were rated on a five-point Likert scale that had 0 (never) as the lowest score and 4 (very often) as the highest score with a total score of 0 to 40 with a high score representing more perceived stress. The reliability of the PSS-10 during this sample was measured through the alpha of Cronbach wherein the value of 0.70 and above would be acceptable.

All data were anonymized using alphanumeric codes and entered into a secure database. Cortisol values and PSS scores were cross-checked to identify and resolve any extreme or erroneous values. Statistical analysis was conducted using SPSS version 26 (IBM Corp., USA). Normality of cortisol levels was assessed using the Shapiro–Wilk test. Paired t-tests were performed for normally distributed data to compare pre- and post-exposure cortisol and PSS-10 scores within groups, while the Wilcoxon signed-rank test was used for non-normal data. Differences between MBBS and BDS students were analyzed using independent t-tests or Mann–Whitney U tests depending on data distribution. A p-value of less than 0.05 was considered statistically significant for all comparisons.

#### Results

# **Participant Characteristics**

One hundred students took part in the research and were divided into 50 MBBS students and 50 BDS students. Both groups were just comparable in demographic and baseline psychological characteristics as demonstrated in Table 1. The average age of the MBBS students (19.9) was not different with BDS students (19.7), p = 0.41. There were also similar gender distribution and baseline stress levels evidenced by the similar pre-exposure PSS scores (15.4 ± 4.2 with MBBS and 16.1 ± 4.6 with BDS). Moreover, there was no significant difference in initial level of salivary cortisol between the two groups, which proved that there was similar physiological stress status before exposure to dissection.

Table 1. Baseline Characteristics of Participants (N = 100)

Variable	MBBS Students	<b>BDS</b> Students	<i>p</i> -value
	(n=50)	(n = 50)	
Age (years), mean $\pm$ SD	$19.9 \pm 1.1$	$19.7 \pm 1.3$	0.41
Gender (M/F)	22/28	20/30	0.68
Baseline PSS Score, mean ± SD	$15.4 \pm 4.2$	$16.1 \pm 4.6$	0.42
Baseline Cortisol (ng/mL), mean $\pm$ SD	$9.82 \pm 2.15$	$10.11 \pm 2.31$	0.51

# **Pre- and Post-Exposure Salivary Cortisol Levels**

After the session of anatomy dissection, the level of cortisol increased significantly in both groups (Table 2). The results were that MBBs students increased to  $14.26 \pm 2.87$  ng/mL as compared to  $9.82 \pm 2.15$  ng/mL and BDS students also had a similar increase to  $15.02 \pm 3.01$  ng/mL as compared to  $10.11 \pm 2.31$  ng/mL. Even though the level was a bit higher in BDS students, the difference between groups was not significant (p = 0.19). The research findings prove that exposing cadavers to arousal produced a considerable physiological stress response across academic programs.

Table 2. Comparison of Salivary Cortisol Levels Pre- and Post-Exposure

Group	Pre-Exposure Cortisol (ng/mL) Mean ± SD	Post-Exposure Cortisol (ng/mL) Mean ± SD	Mean Change	<i>p</i> -value
MBBS $(n = 50)$	$9.82 \pm 2.15$	$14.26 \pm 2.87$	+4.44	< 0.001
BDS $(n = 50)$	$10.11 \pm 2.31$	$15.02 \pm 3.01$	+4.91	< 0.001
Between-Group	_		0.47	0.19
Comparison				

# Perceived Stress Scale (PSS-10) Scores

The same was true of perceived stress scores as Table 3 summarizes. The MBBS and BDS students demonstrated an increment in 15.4 + 4.2 to 21.1 + 4.9 and 16.1 + 4.6 to 22.6 + 5.2 respectively, and these changes were found to be of high statistical significance (p < 0.001). Even though the subjective stress levels were expected to be higher in BDS participants following exposure, the effect of the two groups was not statistically significant (p = 0.11) again. This similar rise of subjective and objective measures of stress proves the psychological intensity of the first cadaver handling.

Table 3. Comparison of Perceived Stress Scores (PSS-10) Before and After Exposure

Group	Pre-Exposure PSS	Post-Exposure PSS	Mean	<i>p</i> -value
	$Mean \pm SD$	$Mean \pm SD$	Change	
MBBS (n = 50)	$15.4 \pm 4.2$	$21.1 \pm 4.9$	+5.7	< 0.001
BDS (n = 50)	$16.1 \pm 4.6$	$22.6 \pm 5.2$	+6.5	< 0.001
<b>Between-Group Comparison</b>	_	_	0.8	0.11

#### **Correlation Between Cortisol Levels and Perceived Stress**

Lastly, Table 4 underscores the positive, although statistically nonsignificant, correlation (r = 0.46, p < 0.001) between post-exposure cortisol and post-exposure PSS scores in all the participants. This shows that the students who had high biochemical stress reaction were also more likely to have higher perceived stress, and therefore the psychological measures used in the study were found to be biologically valid.

Table 4. Correlation Between Post-Exposure Cortisol and PSS Scores

Variable	<b>Correlation Coefficient (r)</b>	<i>p</i> -value
Cortisol vs. PSS Score	0.46	< 0.001

Cortisol levels of both MBBS and BDS students increased considerably after being exposed to cadaveric dissection. There was a parallel increase in perceived stress and the markers of biochemical

stress. There was a statistical insignificance but the BDS students had importance responses to the stressors than the MBBS students. There was a significant relationship between physiological and psychological stress (cortisol).

#### Discussion

This research involved an analysis of the acute physiological and psychological impact of first-time exposure to cadaveric dissection in medical and dental students. Through the simultaneous levels of salivary cortisol and perceived stress levels before and after the dissection, the study will give an overall revelation on the effect of early exposure to formalin-preserved cadavers on neuroendocrine reactions and subjective stress appraisal <sup>11</sup>. These results showed a marked increase in cortisol levels as well as perceived stress right after exposure, which proved that the dissection hall indeed is a highly effective stressor among novice trainees.

The strong growth of salivary cortisol in both MBBS and BDS groups is in agreement with previous accounts of the activation of the hypothalamic-pituitary- adrenal (HPA) axis during emotionally intense or fear inducing exposures to education <sup>12</sup>. Earlier experiments in Europe, Turkey, India and the Middle East have also reported acute increases of salivary cortisol at the early stages of gross anatomy dissection, which is also an extreme physiological arousal by the sensory stimulus presented by chemical odors, visual stimuli and the emotional burden of being exposed to human mortality <sup>13</sup>. Our research confirms these results and also points out the fact that when carefully controlled laboratory conditions, controlled exposure time and preparatory briefing are challenged the stimulus still has importance.

The increase in the perceived stress scores is also a confirmation of the psychological intensity of early dissection experiences. The students have been found to be generally anxious, emotionally uncomfortable, intrusive, sleep disturbed, and physically uncomfortable on their initial cadaver encounter <sup>14</sup>. The same has been witnessed in educational literature where anticipation, fear of the unknown and sociocultural beliefs about death are involved in the cause of early stress peaks. Notably, parallel increase in subjective stress and cortisol in our study is a good indication that the emotional reactions of the students were reflected in the objective biological data, and therefore the PSS-10 is a good measurement tool to reflect real time dissection distress <sup>15</sup>.

Though the mean post exposure cortisol and PSS levels of the BDS students were found to be slightly higher than the MBBS students, they did not differ significantly. However, the tendency is quite significant and can be attributed to a variety of reasons. The dissection experience of dental students is less systematic in the early curriculum, they have lower expectations of practical work, and have less prior exposure to cadaveric material. Also, dental education has been shown to emphasize more on the fine motor skills and chairside interaction at an earlier stage than in other regions, including LMICs, potentially making dental education more emotional when dealing with invasive or visceral material <sup>16</sup>. Even though the differences were not statistically significant, the directionality observed indicates that dental students can make an at-risk group in need of special educational assistance.

The positive but moderate correlation between the levels of cortisol and perceived stress scores post exposure also lends more credence to the explanation that cadaveric dissection does bring about a coherent psychophysiological reaction of stress. As much as the multidimensionality of stress and individual difference is natural, the fact that biological and subjective predictors are congruent indicates that the two spheres are sensitive to the same underlying stimulus. The utility of introducing the objective and subjective monitoring tools into the framework of research in anatomy pedagogy is also supported by this relationship that long encouraged and rarely adopted a dual-measurement implementation strategy.

These findings have significant implications in terms of education. Moderate levels of stress can improve concentration and learning ability, but prolonged stress or extreme stress levels can lead to difficulties in focusing and emotional control as well as poor memory formation. The initial dissection experience may have long-term psychological effects on some students, especially those who are already vulnerable in other ways, such as avoidance behavior, anxiety reinforcement, or lack of interest in anatomy coursework <sup>16</sup>. With the world moving toward humanistic, student-centered

medical education, schools will need to take structured interventions into account in order to reduce stress that they can avoid without undermining educational standards. Examples of such interventions can be pre-dissection programs, psychological orientation sessions, writing about death, mentoring and peer mentoring, mindfulness programs, better ventilation and cadaver preparation, faculty-led discussions about death and professionalism, and staged exposure programs <sup>17</sup>.

Moreover, the results accentuate the cultural contextualization. In most of low- and middle-income countries, social attitudes toward death, donations of body, spiritual, and family factors play a significant role in determining the initial response of the student. Such cultural dimensions might add more emotional distress as compared to the environment where cadaver donation is socialized and promoted via awareness initiatives. Future studies should hence combine the socio-cultural constructs, coping mechanisms and longitudinal follow up to assess the ability to adapt and survive. Another contribution of the study is that it offers some comparative information regarding MBBS and BDS programs <sup>18</sup>. Although the stress patterns between the two groups were not significantly different, the numerical difference was significantly small but consistent in BDS students, which requires further research in terms of curriculum design, previous exposure, and psychological readiness. Multicenter studies with equal sexes and longer follow-up time and qualitative interviews can be considered to elaborate further on the dynamics of stress discipline-specific.

The advantages of this research are a standardized dissection condition, dual points of assessment, application of validated instruments, and both medical and dental cohort. Nevertheless, restrictions are to be recognized <sup>19</sup>. Only acute responses of stress were evaluated and long-term adaptation and effects of repeated exposures were not evaluated. Also, possible confounding factors like chronotype, sleep quality and personality were not studied. And lastly, cortisol is also a strong stress biomarker, but its titer can also be affected by diurnal fluctuation but was controlled by equalizing the time of the sample. All in all, the results confirm that cadaveric dissection does elicit a strong psychophysiological response in first-year medical and dental students. The simultaneous increase in cortisol and perceived stress highlights the simultaneous presence of physiological arousal and emotional strain. The outcomes underscore the relevance of applying wellness-based preparation, positive faculty interaction, and deliberative learning activities to facilitate healthy adaptation in the early anatomy training <sup>20</sup>. The study will inform practice by giving answers to the magnitude of the stress response and the trends in academic programs, which should be implemented to further develop anatomy education and transform it into a more humane, responsive, and scientifically based pedagogical approach.

#### Conclusion

The research shows that cadaveric dissection when performed on the first time triggers a high degree of psychophysiological stress response among MBBS and BDS students. The significant rise in salivary cortisol levels that are accompanied by concomitant increases in the perceived stress scores confirm that the dissection hall is a strong emotional and biological stressor in the initial stages of medical and dental training. Even though there were no significant differences in the responses of both groups, the increased, although not significant, values over the course of BDS students indicate that some subsets might need further preparation.

The positive association between cortisol and perceived stress further confirms the combination of the objective biomarkers and the psychological measures when examining the well-being of the students undergoing anatomy education. These results emphasize the use of guided, student-oriented interventions including orientation programs, psychological coping counselling, better cadaver preparation and reflective learning activities to reduce distress and retain the educational benefits of cadaveric dissection.

#### **Authors' Contributions**

**S.M.** designed the study and supervised the project.

**A.A.** collected data and assisted with analysis.

**A.A.** performed statistical analysis.

H.N.K. conducted laboratory investigations, manuscript editing,.

L.Y. drafted and edited the manuscript.

**N.S.** reviewed the final manuscript and approved it for submission.

All authors approved the final version.

# Acknowledgement

The authors express their sincere gratitude to the participants and the clinical staff who supported data collection and laboratory procedures throughout this study. The team also acknowledges the institutional administration for facilitating the research process.

## **Funding**

No external funding was received for this study. All research activities were carried out with the support of the authors and the host institution.

#### **Conflict of Interest**

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

#### References

- 1. Ko CW, Helzberg JH. Helicobacter pylori infection: Pathophysiology and clinical implications. *Gastroenterology*. 2018;154(3):737–50.
- 2. Kassebaum NJ, Jasrasaria R, Naghavi M, et al. A systematic analysis of global anemia burden. *Blood*. 2014;123(5):615–24.
- 3. Camaschella C. Iron-deficiency anemia. N Engl J Med. 2015;372(19):1832–43.
- 4. World Health Organization. Worldwide prevalence of anaemia 1993–2005. WHO Report. Geneva: WHO; 2008.
- 5. Franchini M, Montagnana M, Lippi G. Helicobacter pylori and iron deficiency anemia. *Hematology*. 2007;12(2):123–32.
- 6. Qu XH, Huang XL, Xiong P, et al. Does Helicobacter pylori infection play a role in iron deficiency anemia? A meta-analysis. *World J Gastroenterol*. 2010;16(7):886–96.
- 7. Hershko C, Ronson A, Souroujon M, et al. Role of H. pylori in iron deficiency anemia. *Am J Hematol*. 2007;82(11):958–65.
- 8. DuBois S, Kearney DJ. Iron-deficiency anemia and Helicobacter pylori infection: A review of evidence. *Am J Gastroenterol*. 2005;100(2):453–9.
- 9. Yip R, Limburg PJ, Ahlquist DA, et al. Helicobacter pylori and recurrent iron deficiency anemia. *J Pediatr Gastroenterol Nutr.* 2004;38(2):146–50.
- 10. Shah S, Bhat N, Ahmed K, et al. Association between H. pylori infection and iron deficiency anemia in adults. *J Coll Physicians Surg Pak*. 2019;29(4):303–7.
- 11. Malik R, Ghoshal UC. H. pylori infection and iron deficiency anemia in India. *Indian J Gastroenterol*. 2011;30(2):67–71.
- 12. Czinn SJ. H. pylori infection and iron deficiency in children. *J Pediatr Gastroenterol Nutr*. 2005;40(1):1–3.
- 13. Annibale B, Capurso G, Lahner E, et al. Concomitant H. pylori infection in iron-deficiency anemia. *Gastroenterology*. 2003;124(3):1031–40.
- 14. Sarker SA, Mahmud H, Ali SM, et al. H. pylori eradication therapy improves iron status in children. *J Pediatr*. 2008;153(5):692–5.
- 15. López-Gómez A, García-Pardo G, Cervera A, et al. H. pylori-associated chronic gastritis and anemia. *Clin Microbiol Infect*. 2017;23(6):379–84.
- 16. DeLoughery TG. Iron deficiency anemia. Med Clin North Am. 2017;101(2):319–32.
- 17. Kato T, Yamaoka Y. Mechanisms of H. pylori-induced iron deficiency. *Clin J Gastroenterol*. 2017;10(3):225–33.

- 18. Vijayvergiya R, Verma S. Iron deficiency anemia and H. pylori infection in young adults. *Trop Gastroenterol*. 2018;39(4):201–6.
- 19. Choe YH, Lee JE, Kim SK. Effect of H. pylori eradication on iron deficiency anemia. *Arch Dis Child*. 2000;82(2):136–40.
- 20. Luther J, Higgins PDR, Schoenfeld PS, et al. Systematic review: H. pylori and iron deficiency. *Aliment Pharmacol Ther*. 2010;32(2):122–32.