

The association between serum cortisol, thyroid profile, paraoxonase activity, arylesterase activity and anthropometric parameters of undergraduate students under examination stress

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Abstract

Stress caused due to examinations during any degree program and at any educational level has been observed at all levels so the present study was conducted to investigate the stress related to final examination in undergraduate students with reference to their academic performance. Fifty-four volunteers were divided into three groups, ($n=18$), according to their academic performance, that is, Group A with low CGPA (≤ 2.99), Group B with average CGPA ($\geq 3.00-3.59$) and Group C with high CGPA (≥ 3.60) and blood samples (3 ml) were taken at three-time intervals, that is, pre-examination, during examination and post examination time interval, at the Department of Physiology, Government College University, Faisalabad. Anthropometric parameters like height, weight, blood pressure, heart rate and body mass index (BMI) were also determined. Blood cortisol and thyroid hormones (triiodothyronine, T3; thyroxine, T4) and thyroid stimulating hormone (TSH)) were measured using ELISA based technique. Arylesterase activity (ARE) and paraoxonase activity (PON-1) were also measured using colorimetric method. The results showed that overall cortisol level was increased at pre-examination and during examination time intervals as compared to the post examination time interval while PON-1 and ARE were significantly ($p < 0.05$) decreased. With regards to T4 and TSH, a non-significant ($p < 0.05$) difference was observed in pre and post examination time interval. It could be concluded from the current study that semester system examinations significantly ($p < 0.05$) imposed stress in all students group at pre-examination interval as compare to post examination interval and this examination related stress was find more in the students already obtained low CGPA as compared to the other studied groups. Although, a correlation between academic performance and examinations stress in semester system was found but a large scale trial in multiple institutions throughout the Pakistan is required to exclude any socioeconomic factors because Pakistan belongs to developing country.

Keywords

academic performance, arylesterase, cortisol, examination related stress, paraoxanase, thyroid hormones

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Introduction

Stress can lead to “flight or fight,” response that can cause endocrinological and physiological maladjustments. The presence of stress in students has come under analysis as it can lead to short term or long term psychological and physical health related problems.^{1,2} Examination related stress is a physiological and emotional response of students to the prospect of participating in examinations.³ Within the context of students, stress is most commonly observed during the examinations and it often reaches its threshold just before the start of examinations.⁴ The incidence of examination stress has increased from 27% in 2009 to 40% in 2010 among students undergoing examinations.⁵ Students entering competitive environment of universities from protected environment of home and college, feel immense pressure to perform.⁶ In some cases, the examination related stress can result in a vicious cycle of low self-esteem, poor academic performance, panic attacks, anxiety and depression.^{7,8} Stress causes physical and emotional effects as well as cognitive symptoms such as palpitations, fatigue, headaches, restlessness, nightmares, difficulty in concentration, and difficulty in organizing thoughts.^{1,9} Mild stress may be temporarily helpful in performance and may give benefit a person in healthy competition during examination, while continuous stress what so ever the intensity can potentially lead to serious anxiety and depression. High stress levels also renders great difficulties in focusing on a particular task.^{3,10–12}

Psychological stress has short-term as well as long-term effects. In short term effect, the basal cortisol level and secretion of corticotrophin releasing hormone increases so that the body adapts itself to a hyper catabolic condition.³ Cortisol is a steroid hormone produced in response to the adrenocorticotrophic hormone (ACTH) and is the end product of hypothalamic-pituitary-adrenal (HPA) axis.^{13–15} Cortisol hormone is mostly used in research as an established physiological measure of stress and its levels reflect psychological stress. During stressful conditions, the body increases the production of cortisol from adrenal cortex.¹⁴ It is also linked with many psychological and physiologic processes like learning and memory, cell death, neural development, metabolism, and immune function.¹⁵ The cortisol is release by slow peripheral response to acute stress and it is proposed that it has both suppressive and stimulatory roles to body stress.¹⁶ Chronic stress is associated

with high cortisol levels, but it is a matter of open discussion whether cortisol is cause for these physiological stress responses or produced in reaction to chronic stressors.¹⁷ The central nervous system (CNS) plays a vital role in psychological and physical processes and physiological response to a stressor. Psychological and physical stress responses are characterized by the triggering of HPA axis which releases cortisol and activation of autonomic nervous system (ANS) which releases catecholamine. The sympathetic part of the ANS is responsible for different physiological responses such as change in heart and respiratory rate, blood flow to muscles.^{18,19} Raised levels of cortisol affect learning skills and recollection, increase weight, heart diseases, increase cholesterol as well as decreased resilience and mental illness—particularly in young people.^{2,20,21}

Stress also rises the metabolic burdens of body and may alter the hypothalamic-pituitary-thyroid (HPT) axis and thus change hormone levels including the thyroid status.²² Thyroxine (T4) is an important hormone of the body and many researchers have found a relationship of stress with the thyroid diseases.²³ Level of thyroglobulin can be used to notice damage to thyroid gland.^{3,24} Thyroid hormone activities can be altered by cortisol levels but relationship between thyroid hormone levels and stress in peripheral blood have produced mixed results, that is, showing that a slight increase or no change in hormone levels takes place in response to mild stress or a decrease takes place after high stress.^{25,26}

Oxidants are formed in both normal physiological as well as pathological conditions, such as hydrogen peroxide, protein peroxide, lipid hydroperoxide, and peroxy nitrates. The brain consumes large amount of oxygen and has a weak antioxidant defenses therefore brain is prone to oxidative damage. Constant oxidative damage to brain lead to development of another depressive episode in person already suffering from depressive episodes.²⁷ Paraonase (PON1) enzyme related with paraonase and arylesterase activities and protects the body from oxidative stress. PON1 is an antioxidant bio-scavenger that responsible for lipid peroxides hydrolysis and also a main factor in the antioxidant system. Several research studies exposed that if oxidative stress become increased it can cause reduction in PON1 activity.^{28,29} Deficiency in these activities may lead to anxiety that the diagnosed marker of generalized anxiety disorder.³⁰ It has

been found that paraoxonase activity become decrease in the patient of insomnia and vitiligo.^{31,32} As far as we could possibly know, PON and ARE activities have not been examined as yet in examination stress. The present study was conducted to explore the stress level in the students by measuring the cortisol, paraoxonase activity and thyroid levels at different phases, that is, pre, during, and post examination intervals.

Material and methods

Study plan and collection of blood samples

Comparative cross-sectional study was undertaken using stratified random sampling technique at Health Biology Research Lab, Department of Physiology, Government College University, Faisalabad-Pakistan. A Sample size of 54 undergraduate candidates was selected from 6th semester (3rd year undergraduate students) of Department of Physiology, Government College University, Faisalabad and divided into three groups according to their previous academic performance as Group A with low CGPA (≤ 2.99) students, Group B with average CGPA (3.00–3.59) students and Group C with high CGPA (≥ 3.60). After written consent from all volunteers (both males and females), 3 ml of blood sample was drawn at three time intervals, that is, pre-examination (1 h before conducting the examination), during examination and post examination interval (after 2 days of final examinations), then centrifuged at $2000 \times g$, for 5 min to separate the serum from cellular part of blood. The study was conducted after the approval of Institutional Review Board (Ref. No. GCUF/ERC/149). As no drug or medication was used so trial registration was not applicable for this study.

Inclusion and exclusion criteria

The candidates included in this study were both males and female with ages between 20 and 25 years and were not on any kind of medication or suffering from any neurological or psychological problems. Students assessed for neurological or psychological problems by observing their previous behavior, by taking medical history and examine the physical activities. All the students included were also confirmed that they enrolled the same credit hours for current study. Student's enrolled extra credit hours as well as enrolled any supplementary subject were excluded.

Anthropometric measurements

Anthropometric variables such as age, weight, height, and body mass index (BMI) were assessed. A questioner was filled by participant students that explain the age of students and confirmed from their national identity card. Weight as well as height of every individual was measured with the help of standard weighing machine and standard height measuring scale, respectively. BMI was determined by using formula: weight (kg)/Height (m^2). Blood pressure was measured by aneroid sphygmomanometer and stethoscope and heart rate was measured by conventional method using radial artery.

Serum hormones

Cortisol (nmol/L). Blood cortisol in serum was determined by competitive enzyme immunoassay (CEIA) method using commercially available cortisol ELISA kit supplied by AccuBind® (Monobind Inc. Lake Forest, CA 92630 USA). The sensitivity range of the kit was 156–10,000 pg/ml and absorbance of the serum was taken at 405 nm. The precision CV value was $< 10\%$.

Thyroid hormones (U/ml). Triiodothyronine (T3) and Thyroxine (T4) levels in serum were determined by enzyme immunoassay (EIA) method using commercially available T3 and T4 ELISA kit supplied by DiaMetra® (Perugia Italy) while thyroid stimulating hormone (TSH) in serum was determined by enzyme immunoassay (EIA) method using commercially available TSH ELISA kit supplied by Calbiotech, Inc. (El Cajon, CA 92020 USA).

Stress markers

Paraoxonase activity (PON-1; U/min/ml). The paraoxonase enzyme (PON-1) activity was determined by method as described by Nisar et al.³³ PON-1 activity was measured by using 2 mmol/L paraoxon as the assay substrate. The minimum detection limit of this assay was 80–100 U/min/L. The hydrolysis sensitivity rate was stable up to 5 min and the intra-assay CV was $< 10\%$.³³

Arylesterase activity (ARE; KU/min /L). The arylesterase enzyme activity per minute was determined by method previously defined by Nisar et al.³³ Reaction mixture comprised of 2 mmol/L phenyl acetate

with 2 mmol/L CaCl₂ in 0.1 M/L Tris-HCL Buffer. The minimum detection limit of this assay was 40–55 KU/min/L and intra-assay CV was <7%.³³

Statistical analysis

The results were represented as mean \pm SEM and were analyzed using SPSS statistical software (trial version 20). For analysis purpose, one-way ANOVA was used to compare CGPA and examination time interval group. Two-way ANOVA to find interaction of examination interval and CGPA on different parameters was also conducted. Post-hoc test (Tukey) was used to find significant difference between groups. A level of $p \leq 0.05$ was taken as statistically significant.

Results

Anthropometric parameters

Different anthropometric parameters like age, height, weight, and body mass index (BMI) were compared and shown in Tables 1 and 2. Heart rate during-examination interval was significantly decreased ($p < 0.01$) from pre-examination interval (Table 1). While, HR was increased in the post examination interval. Systolic and diastolic blood pressure was increased in group A as compared to group B and C during the pre-examination interval. Overall mean of systolic and diastolic blood pressure was significantly increased during the pre-examination interval as compared to the post-examination interval. BMI results showed the non-significant ($p > 0.05$) result during different examination intervals.

Serum hormones

Results showed that cortisol level in post-examination interval was significantly ($p < 0.01$) decreased as compared to both pre-examination interval and during-examination interval while a non-significant ($p > 0.05$) difference was reported between pre-examination and during-examination interval (Figure 1). The overall mean of cortisol showed significant ($p < 0.05$) increase in group C among all groups and moreover, it was also reported that at post-examination interval cortisol level was lowest. The result showed non-significant interaction of T₃, T₄, and TSH between examination intervals and CGPA as well as overall means

Table 1. Comparison of anthropometric parameters with reference to examination time intervals.

| Anthropometric parameters | Intervals | N | Mean \pm S.E |
|---------------------------|-------------|----|-------------------------------|
| Age (years) | Pre-exam | 54 | 20.91 \pm 0.19 |
| | During-exam | 54 | 20.91 \pm 0.19 |
| | Post-exam | 54 | 20.91 \pm 0.19 |
| Height (cm) | Pre-exam | 54 | 161.56 \pm 1.75 |
| | During-exam | 54 | 161.56 \pm 1.84 |
| | Post-exam | 54 | 161.56 \pm 1.69 |
| Weight (Kg) | Pre-exam | 54 | 53.06 \pm 2.66 |
| | During-exam | 54 | 53.00 \pm 2.60 |
| | Post-exam | 54 | 53.06 \pm 2.68 |
| DBP (mmHg) | Pre-exam | 54 | 80.00 \pm 2.14 |
| | During-exam | 54 | 78.33 \pm 2.02 |
| | Post-exam | 54 | 75.56 \pm 1.45 |
| SBP (mmHg) | Pre-exam | 54 | 116.11 \pm 2.00 |
| | During-exam | 54 | 112.22 \pm 1.73 |
| | Post-exam | 54 | 111.67 \pm 2.18 |
| HR (Beats/minute) | Pre-exam | 54 | 74.83 \pm 3.50 ^a |
| | During-exam | 54 | 60.00 \pm 1.22 ^b |
| | Post-exam | 54 | 76.78 \pm 2.38 ^a |
| BMI (kg/m ²) | Pre-exam | 54 | 20.19 \pm 0.80 |
| | During-exam | 54 | 20.24 \pm 0.80 |
| | Post-exam | 54 | 20.11 \pm 0.81 |

N: no. of participants; SE: standard error; DBP: diastolic blood pressure; SBP: systolic blood pressure; HR: heart rate; BMI: body mass index, Values sharing different alphabets (a, b as superscript) are significantly ($p < 0.05$) different between pre, during and post exam for a parameter.

(Figure 2(a)–(c)). However, the overall mean of T₄ showed significant increase in group C from group B and group A while there was non-significant difference in examination intervals (Figure 2(a)–(c)). On the other hand, the overall mean of TSH showed significant decrease in group A from group B and group C.

Stress markers

The result of paraoxanase activity showed significant difference between examination interval. The overall mean of paraoxanase activity was significantly low in pre-examination interval as compared to the post-examination interval (Figure 3). Similarly, overall mean of arylesterase activity was also decreased significantly during pre-examination interval as compared to the post-exam interval (Figure 4). The overall mean of ARE showed significant difference in group C from group B and group A, while pre-examination interval, during-exam interval and post-exam interval showed significant difference from one another.

Table 2. Comparison of anthropometric parameters with reference to CGPA groups.

| Anthropometric parameters | Groups | N | Mean \pm S.E |
|---------------------------|---------|----|--------------------------------|
| Age (years) | Group A | 18 | 20.67 \pm 0.12 |
| | Group B | 18 | 20.83 \pm 0.17 |
| | Group C | 18 | 21.25 \pm 0.24 |
| Height (cm) | Group A | 18 | 167.50 \pm 1.70 |
| | Group B | 18 | 161.41 \pm 0.80 |
| | Group C | 18 | 155.97 \pm 1.36 |
| Weight (Kg) | Group A | 18 | 62.72 \pm 2.39 ^a |
| | Group B | 18 | 50.61 \pm 2.27 ^b |
| | Group C | 18 | 45.78 \pm 1.07 ^c |
| DBP (mmHg) | Group A | 18 | 80.00 \pm 2.56 ^a |
| | Group B | 18 | 76.67 \pm 1.61 ^b |
| | Group C | 18 | 77.22 \pm 1.35 ^b |
| SBP (mmHg) | Group A | 18 | 117.78 \pm 2.49 ^a |
| | Group B | 18 | 111.11 \pm 1.37 ^b |
| | Group C | 18 | 111.11 \pm 1.59 ^b |
| HR (Beats/minute) | Group A | 18 | 71.33 \pm 3.49 |
| | Group B | 18 | 69.89 \pm 3.24 |
| | Group C | 18 | 70.39 \pm 2.56 |
| BMI (kg/m ²) | Group A | 18 | 22.34 \pm 0.82 |
| | Group B | 18 | 19.31 \pm 0.73 |
| | Group C | 18 | 18.90 \pm 0.55 |

N: no. of participants; SE: standard error; Group A: CGPA \leq 2.99; Group B: CGPA 3.00–3.59, Group; C: CGPA \geq 3.60; DBP: diastolic blood pressure; SBP: systolic blood pressure; HR: heart rate; BMI: body mass index, Values sharing different alphabets (a-c as superscript) are significantly ($p < 0.05$) different between groups for a parameter.

Discussion

Examination stress during education is a common observation in student life. Some consider examination stress as “time bomb” for mental health³⁴ while other consider it as an opportunity.³⁵ Heart Rate among various students showed significant results during different time-intervals of examination. In pre-examination interval, HR was 74.83 \pm 3.50 bpm, it decreased to 60.00 \pm 1.22 bpm during-examination interval which showed that students are coming in relaxed condition or less stressed condition but in post-examination interval, HR increased to 76.78 \pm 2.38 bpm which was surprising but it could be due to thinking of results or some other factors. With regards to systolic blood pressure (SBP) and diastolic blood pressure (DBP) the variations were non-significant ($p > 0.05$). Sharma and Wavare⁴³ conducted study on medical students and found significant difference in SBP, DBP, and HR due to examination stress. While Clarke et al.⁴⁴ found no link between pre-examination stress and mean heart rate while exercise was linked with significant heart rate elevation. In current study, HR

was observed to be non-significantly different in different groups. Similarly, study by Harvey et al.⁴⁵ found no significance in increased heart rate between low and high stress situations. Cortisol (glucocorticoid) derived from cholesterol, acted as steroid hormone synthesized in adrenal glands and is mainly involved in stress response.³⁶ In the current study cortisol showed significant decrease during different intervals of examinations. In pre-examination interval, cortisol was 36.79 \pm 0.01 nmol/L which decreased ever so slightly to 36.78 \pm 0.01 nmol/L during-examination interval and dropped to 28.07 \pm 1.50 nmol/L in post-examination interval. This drop in cortisol showed decrease in psychological stress. Potey et al.³⁷ compared the effect of examination stress on cortisol level in control group and study group that doing regular yoga. They found that cortisol hormone level decreased significantly in study group doing regular yoga. Another similar study conducted by Rajak et al.³⁸ had shown a correlation between significantly decreased levels of cortisol and reduced stress. Ehiaghe et al.³⁹ assessed serum cortisol in young male postgraduate students and found a significant increase in serum cortisol at 1st day of semester when compared with midway of semester and on last examination morning. These results show that cortisol levels increase in stressful conditions and when stressful event ends, cortisol level resume their normal levels. In current study cortisol level was high during examinations and decreased after the end of examinations (post-examination interval).

Current study showed cortisol was released in excess quantity (36.69 \pm 0.06 nmol/L) in students with high grading while the amount of cortisol in average and low grading students were approximately same (32.46 \pm 1.51 nmol/L and 32.49 \pm 1.49 nmol/L, respectively). This result was opposite to expectations as high grade students had high values of cortisol than average and low grade students. No previous study has been conducted in this regard. The current study also showed significant difference in release of TSH and T4 with highest values (3.85 \pm 0.67 mIU/L and 12.47 \pm 0.22 μ g/dL, respectively) in high grade students and least values (1.57 \pm 0.32 mIU/L and 11.87 \pm 0.14 μ g/dL, respectively) in low grade students. Previous studies showed variable results in this regard. Emotional and physical stresses might have different and contrasting effects.⁴⁰ In previous studies, increased secretion of thyroid hormone levels in serum had been observed in stressed animals and in

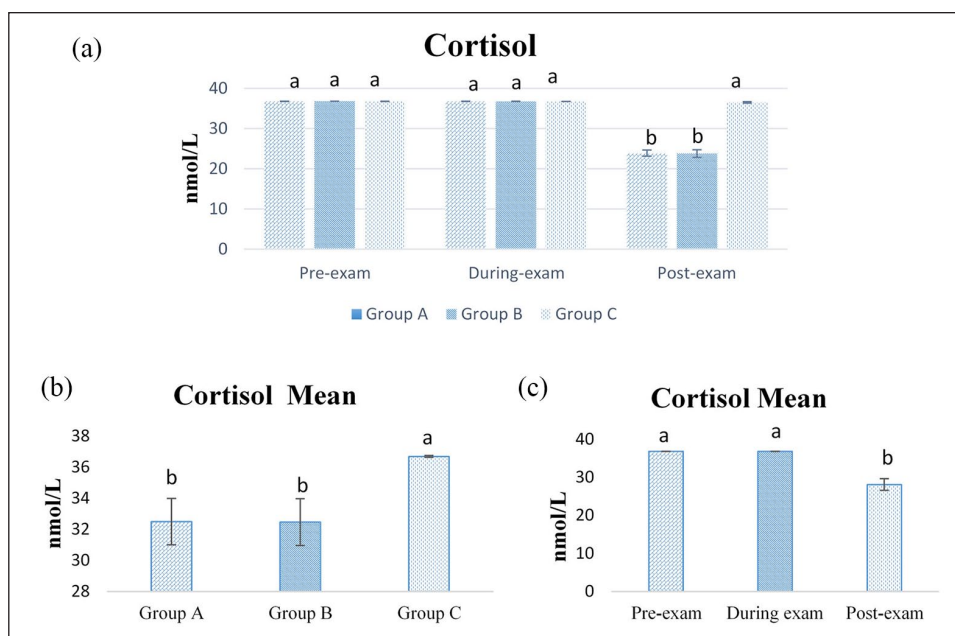


Figure 1. Comparison of serum cortisol level with reference to examination time intervals: (a) the mean cortisol (COR) \pm SE (nmol/L) of different CGPA groups at various examination intervals, (b) the overall mean COR \pm SE (nmol/L) irrespective of examination intervals, and (c) the overall mean COR \pm SE (nmol/L) irrespective of CGPA groups. COR: cortisol, low CGPA (≤ 2.99) students, group B with average CGPA (≥ 3.00 – 3.59) students and group C with high CGPA (≥ 3.60). Means sharing similar letter are statistically non-significant ($p \geq 0.05$).

acute psychiatric patients on admission as well in medical students during examinations. Cheserek et al.⁴¹ conducted study to compare thyroid hormones level in control mice with high fat diet (HFD) and found increased level of TSH and T4 while decreased level of T3 in HFD mice as compare to control mice. While another studies conducted by Helmreich et al.,⁴² Helmreich and Tylee²⁵ on rat model and review article by Ranabir and Reetu²² showed decreased level of TSH and T4 but no effect on T3 levels.

Paraoxanase and arylesterase activities also showed the significant result during pre-examination interval and post examination interval. During stress, PON and ARE activities were significantly decreased within the group. The result of the study showed a significant reduction in the paraoxanase and arylesterase activities during pre-examination interval as compared to the post-examination interval. Ozturk et al.,⁴⁶ reported that the paraoxanase activity has been decreased during the exercise when oxidative stress was increased. Life style and genetic factors also effect the activity of paraoxanase. Paraoxanase and arylesterase activity decreased in the case of different diseases caused

by oxidative stress including hypothyroidism, cardiac disease and acute coronary syndrome^{28,47,48} The result of the present study showed the examination stress can lead to decrease the activity of paraoxanase and aryl esterase enzymes.

Limitations of research

Although the results of current study revealed significant correlation in examination related stress in University students under semester system. However, Sample size is very small (54 students) because students from the same Standard/Class (6th semester) were selected to avoid any age related as well as class related variations in results so number of samples is the limitation of this study. Moreover, a large scale trial in multiple institutions throughout the Pakistan is required to exclude any socioeconomic factors related stress in students because Pakistan belongs to developing country. So by taking this baseline study we could extend this research throughout Pakistan in educational institutes with different examination systems and having students from different socioeconomic status.

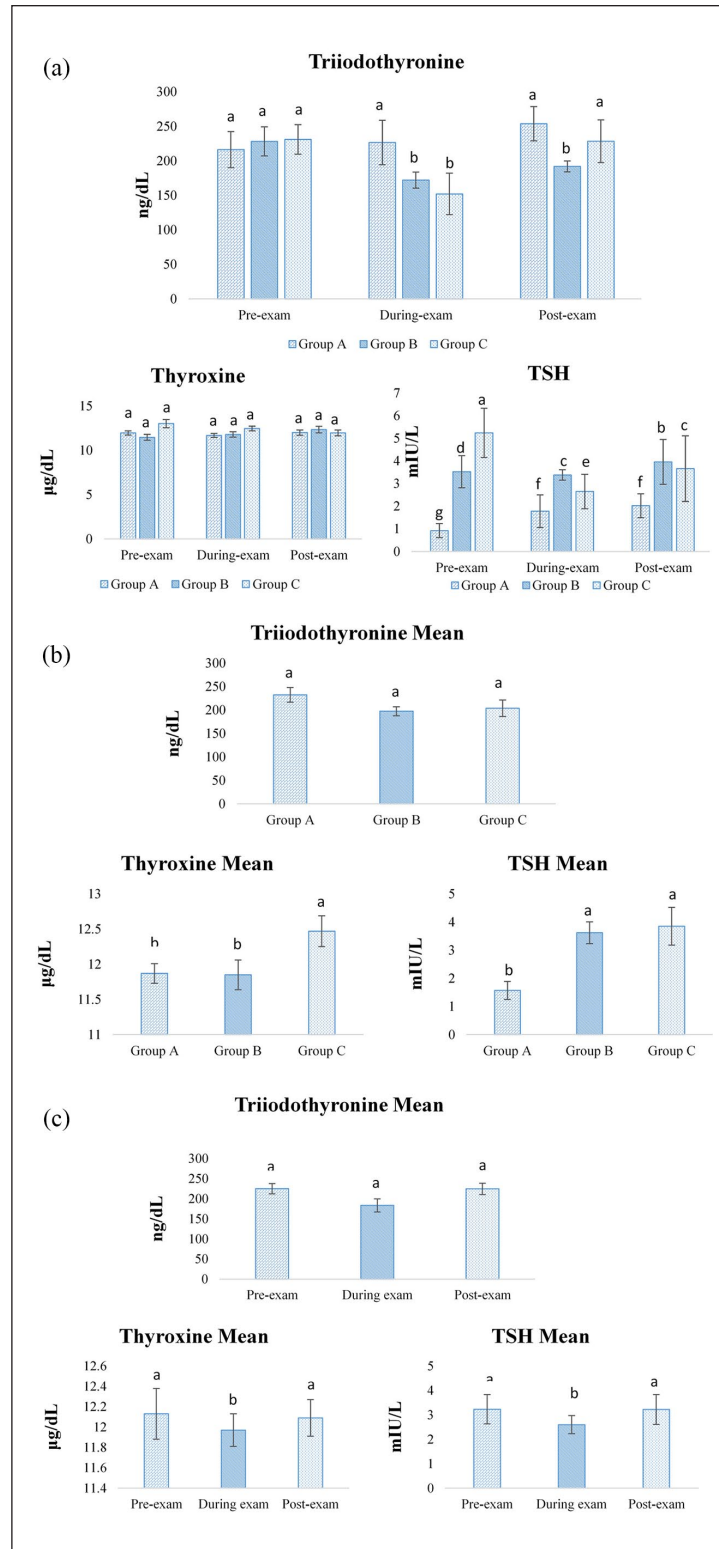


Figure 2. (a) The mean thyroid hormone \pm SE of different CGPA groups at various examination intervals. TSH: thyroid stimulating hormone, low CGPA (≤ 2.99) students, group B with average CGPA (≥ 3.00 – 3.59) students and group C with high CGPA (≥ 3.60). Means sharing similar letter are statistically non-significant ($p \geq 0.05$), (b) overall mean \pm SE Thyroid hormone irrespective of examination intervals. Group A low CGPA (≤ 2.99) students, group B with average CGPA (≥ 3.00 – 3.59) students and group C with high CGPA (≥ 3.60). Means sharing similar letter are statistically non-significant ($p \geq 0.05$), and (c) overall mean \pm SE Thyroid hormone irrespective of CGPA groups. TSH: thyroid stimulating hormone, means sharing similar letter are statistically non-significant ($p \geq 0.05$).

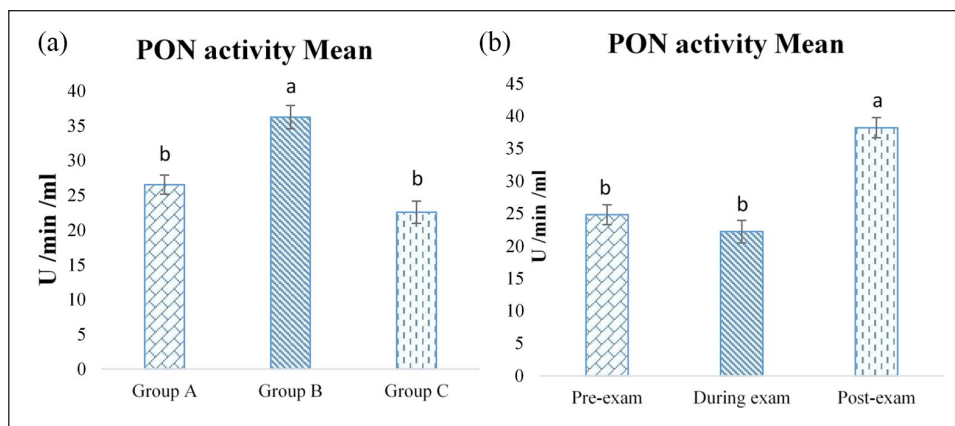


Figure 3. Comparison of serum Paraoxanase activity with reference to examination time intervals: (a) the mean PON \pm SE (U/min/ml) irrespective of examination intervals and (b) the mean PON \pm SE (U/min/ml) irrespective of CGPA. Group A low CGPA (≤ 2.99) students, group B with average CGPA (≥ 3.00 – 3.59) students and group C with high CGPA (≥ 3.60). Means sharing similar letter are statistically non-significant ($p \geq 0.05$).

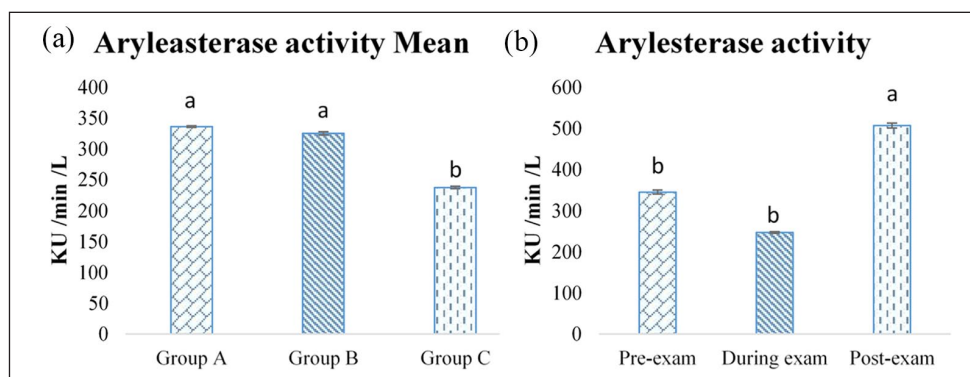


Figure 4. Comparison of serum Arylesterase activity with reference to examination time intervals: (a) the mean ARE \pm SE (KU/min/L) irrespective of examination intervals and (b) the mean ARE \pm SE (KU/min/L) irrespective of CGPA groups. ARE: Arylesterase, low CGPA (≤ 2.99) students, group B with average CGPA (≥ 3.00 – 3.59) students and group C with high CGPA (≥ 3.60). Means sharing similar letter are statistically non-significant ($p \geq 0.05$).

Conclusion

In conclusion, the results give confidence to the observations that examination stress was high in the students with low CGPA as compared to the other studied groups. Examination stress in the students increased the cortisol level and also effects the heart rate, blood pressure, depressed the enzymatic activities of paraoxanase and arylesterase. Overall results explored that examination related stress in students was high in all groups particularly in the students already have low GCPA at pre examination interval as compare to the post examination interval. Although, a correlation between academic performance and examinations stress in semester system was found but a large scale trial in multiple institutions throughout the Pakistan is

required to exclude any socioeconomic factors because Pakistan belongs to developing country.

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Ethics approval

All procedures were performed at Department of Physiology, Government College University, Faisalabad which were approved by the Institutional Review Board (Ref. No. GCUF/ERC/149).

Informed consent

Written informed consent was obtained from all subjects before the study.

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References

- Mittal R and Kumar R (2018) Exam stress in MBBS students and the methods used for its alleviation. *International Journal of Medical and Dental Sciences* 7: 1604–1608.
- Abidin Z, Syafiq A, Rahim RA et al. (2017) Current and potential developments of cortisol aptasensing towards point-of-care diagnostics (POTC). *Sensors* 17(5): 1180.
- Hettiarachchi M, Lakmal Fonseka C, Gunasekara P et al. (2014) How does the quality of life and the underlying biochemical indicators correlate with the performance in academic examinations in a group of medical students of Sri Lanka?. *Medical Education Online* 19(1): 22772.
- Pisanski K, Nowak J and Sorokowski P (2016) Individual differences in cortisol stress response predict increases in voice pitch during exam stress. *Physiology & Behavior* 163: 234–238.
- Mohapatra S, Panigrahi SK and Rath D (2012) Examination stress in adolescents. *Asian Journal of Paediatric Practice* 16(1): 1–3.
- Ibrahim MB and Abdelreheem MH (2015) Prevalence of anxiety and depression among medical and pharmaceutical students in Alexandria University. *Alexandria Journal of Medicine* 51(2): 167–173.
- Jafari N, Loghmani A and Montazeri A (2012) Mental health of medical students in different levels of training. *International Journal of Preventive Medicine* 3(Suppl1): S107.
- Thangaraj S and D'souza L (2014) Prevalence of stress levels among first year medical undergraduate students. *International Journal of Interdisciplinary and Multidisciplinary Studies* 1(5): 176–181.
- Sujatha B and Subhalakshmi S (2016) Effect of stress on exam going first year medical students of Tirunelveli. *International Journal of Medical Research & Health Sciences* 5(1): 118–121.
- Afrisham R, Sadegh-Nejadi S, SoliemaniFar O et al. (2016) Salivary testosterone levels under psychological stress and its relationship with rumination and five personality traits in medical students. *Psychiatry Investigation* 13(6): 637.
- Nakhaee A, Shahabzadeh F and Erfani M (2013) Protein and lipid oxidative damage in healthy students during and after exam stress. *Physiology & Behavior* 118: 118–121.
- Singh R, Goyal M, Tiwari S et al. (2012) Effect of examination stress on mood, performance and cortisol levels in medical students. *Indian Journal of Physiology and Pharmacology* 56(1): 48–55.
- Brown NJ, Kimble RM, Rodger S et al. (2014) Biological markers of stress in pediatric acute burn injury. *Burns* 40(5): 887–895.
- Mizawa M, Yamaguchi M, Ueda C et al. (2013) Stress evaluation in adult patients with atopic dermatitis using salivary cortisol. *BioMed Research International* 2013: 138027.
- Shirtcliff EA, Buck RL, Laughlin MJ et al. (2015) Salivary cortisol results obtainable within minutes of sample collection correspond with traditional immunoassays. *Clinical Therapeutics* 37(3): 505–514.
- Andersson H, Tullberg C, Ahrné S et al. (2016) Oral Administration of *Lactobacillus plantarum* 299v reduces cortisol levels in human saliva during examination induced stress: A randomized, Double-Blind Controlled Trial. *International Journal of Microbiology* 2016: 8469018.
- Cohen S, Janicki-Deverts D, Doyle WJ et al. (2012) Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. *Proceedings of the National Academy of Sciences of the United States of America* 109(16): 5995–5999.
- Skoluda N, Strahler J, Schlotz W et al. (2015) Intra-individual psychological and physiological responses to acute laboratory stressors of different intensity. *Psychoneuroendocrinology* 51: 227–236.
- Vanaelst B, Michels N, Clays E et al. (2014) The association between childhood stress and body composition, and the role of stress-related lifestyle factors—cross-sectional findings from the baseline ChiBS survey. *International Journal of Behavioral Medicine* 21(2): 292–301.
- Lindau M, Almkvist O and Mohammed AH (2016) Effects of stress on learning and memory. In: George F (ed) *Stress: Concepts, cognition, emotion, and behavior*. San Diego, CA: Academic Press, pp.153–160.
- Yuan Y, Leung AW, Duan H et al. (2016) The effects of long-term stress on neural dynamics of working memory processing: An investigation using ERP. *Scientific Reports* 6(1): 1–10.

22. Ranabir S and Reetu K (2011) Stress and hormones. *Indian Journal of Endocrinology and Metabolism* 15(1): 18.
23. Tsatsoulis A (2006) The role of stress in the clinical expression of thyroid autoimmunity. *Annals of the New York Academy of Sciences* 1088(1): 382–395.
24. Czarnocka B (2011) Thyroperoxidase, thyroglobulin, Na (+)/I (-) symporter, pendrin in thyroid autoimmunity. *Frontiers in Bioscience (Landmark edition)* 16: 783–802.
25. Helmreich DL and Tylee D (2011) Thyroid hormone regulation by stress and behavioral differences in adult male rats. *Hormones and Behavior* 60(3): 284–291.
26. Sun Q, Liu A, Ma Y et al. (2016) Effects of forced swimming stress on thyroid function, pituitary thyroid-stimulating hormone and hypothalamus thyrotropin releasing hormone expression in adrenalectomy Wistar rats. *Experimental and Therapeutic Medicine* 12(5): 3167–3174.
27. Moylan S, Maes M, Wray NR et al. (2013) The neuroprogressive nature of major depressive disorder: Pathways to disease evolution and resistance, and therapeutic implications. *Molecular Psychiatry* 18(5): 595–606.
28. Cebeci E, Alibaz-Oner F, Usta M et al. (2012) Evaluation of oxidative stress, the activities of paraoxonase and arylesterase in patients with subclinical hypothyroidism. *Journal of Investigative Medicine* 60(1): 23–28.
29. Cebeci E, Oner FA, Usta M et al. (2011) Evaluation of oxidative stress, the activities of paraoxonase and arylesterase in patients with subclinic hypothyroidism. *Acta Bio-Medica: Atenei Parmensis* 82(3): 214–222.
30. Bulut M, Selek S, Bez Y et al. (2013) Reduced PON1 enzymatic activity and increased lipid hydroperoxide levels that point out oxidative stress in generalized anxiety disorder. *Journal of Affective Disorders* 150(3): 829–833.
31. Liang B, Li YH and Kong H (2013) Serum paraoxonase, arylesterase activities and oxidative status in patients with insomnia. *European Review for Medical and Pharmacological Sciences* 17(18): 2517–2522.
32. Yesilova Y, Turan E, Ucmak D et al. (2012) Reduced serum paraoxonase-I levels in vitiligo: Further evidence of oxidative stress. *Redox Report* 17(5): 214–218.
33. Nisar J, Mustafa I, Anwar H et al. (2017) Shiitake culinary-medicinal mushroom, *Lentinus edodes* (Agaricomycetes): A species with antioxidant, immunomodulatory, and hepatoprotective activities in hypercholesterolemic rats. *International Journal of Medicinal Mushrooms* 19(11): 981–990.
34. Hough A (2011) Middle-class student exam stress ‘creating mental health time bomb’. *The Telegraph*, May 21, <http://www.telegraph.co.uk/education/educationnews/8527066/Middle-class-student-exam-stress-creating-mental-health-time-bomb.html> (accessed 12 May 2012).
35. Strack J and aqnd Esteves F (2015) Exams? Why worry? Interpreting anxiety as facilitative and stress appraisals. *Anxiety, Stress, & Coping* 28(2): 205–214.
36. MacLaughlin BW, Wang D, Noone AM et al. (2011) Stress biomarkers in medical students participating in a mind body medicine skills program. *Evidence-Based Complementary and Alternative Medicine* 2011: 950461.
37. Potey GG, Rahul V, Chanda R et al. (2016) Effect of yoga practices on examination stress induced changes in serum cortisol level & cardiovascular parameters in young healthy medical students. *World Journal of Pharmacy and Pharmaceutical Sciences* 5(6): 1902–1915.
38. Rajak C, Verma R, Singh P et al. (2016) Effect of yoga on serum adrenaline, serum cortisol levels and cardiovascular parameters in hyper-reactors to cold pressor test in Young healthy volunteers. *European Journal of Pharmaceutical and Medical Research* 3(8): 496–502.
39. Ehiaghe FA, Agbonlahor DE, Ositadima MI et al. (2014) Effect of academic stress on serum cortisol level and CD4 cell count in young male postgraduate students in Okada, Nigeria. *International Journal of Biological and Chemical Sciences* 8(3): 1249–1253.
40. Sarne D (2016) Effects of the environment, chemicals and drugs on thyroid function. In: Kenneth RF (ed) *Endotext*. South Dartmouth, MA: MDText. com, Inc.
41. Cheserek MJ, Wu G, Li L et al. (2016) Cardioprotective effects of lipoic acid, quercetin and resveratrol on oxidative stress related to thyroid hormone alterations in long-term obesity. *The Journal of Nutritional biochemistry* 33: 36–44.
42. Helmreich DL, Parfitt DB, Lu XY et al. (2005) Relation between the hypothalamic-pituitary-thyroid (HPT) axis and the hypothalamic-pituitary-adrenal (HPA) axis during repeated stress. *Neuroendocrinology* 81(3): 183–192.
43. Sharma B and Wavare R (2013) Academic stress due to depression among medical and para-medical students in an Indian medical college: Health initiatives cross sectional study. *Journal of Health Sciences* 2013; 3(5): 29–38.
44. Clarke S, Horeczko T, Cotton D et al. (2014) Heart rate, anxiety and performance of residents during a simulated critical clinical encounter: A pilot study. *BMC Medical Education* 14(1): 153.
45. Harvey A, Bandiera G, Nathens AB et al. (2012) Impact of stress on resident performance in simulated

- trauma scenarios. *Journal of Trauma and Acute Care Surgery* 72(2): 497–503.
46. s H, Gungor M, Yigit S et al. (2009) Effect of exercise on the paraoxonase activity. *Clinical Biochemistry* 42(4–5):343.
47. Kabaroglu C, Mutaf II, Boydak B et al. (2004) Association between serum paraoxonase activity and oxidative stress in acute coronary syndromes. *Acta Cardiologica* 59(6): 606–611.
48. Gur M, Yildiz A, Demirbag R et al. (2007) Paraoxonase and arylesterase activities in patients with cardiac syndrome X, and their relationship with oxidative stress markers. *Coronary Artery Disease* 18(2): 89–95.