Effect of Academic Stress on Physiological and Biochemical Parameters among Medical Students

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ABSTRACT

Objective

The primary aim of this study was to understand the effects of a stressed academic environment in medical students through evaluating different physical and biochemical parameters.

Methodology

This study is longitudinal and based on a convenient sampling method, including 50 volunteer medical students of Ayub Medical College, Abbottabad with mean age 21-25yrs (24±1) in both stressed and unstressed states. Physical and biochemical parameters were evaluated by measuring serum Cortisol and plasma Serotonin levels by using ELISA kits. Data were analyzed by using SPSS-22.

Results

Results showed an insignificant difference of Serum Cortisol (6.6±4.2ug/dl) in stressed and (7.0±4.7ug/dl) unstressed states with (p=0.72). , plasma serotonin values in stressed (149±140ng/ml) and unstressed (125±108ng/ml) with (p =.47) Parameters like blood pressure (both systolic and diastolic) with a p-value of <.01 showed significant difference during the examination. However, other variables like Age, Height, Weight, BMI (p= .946), Heart Rate (p= .149) showed no significant difference.

Conclusion

The study concludes that little to moderate levels of changes of serum cholesterol and plasma serotonin values may occur due to the academic pressure on students, which can affect their professional capabilities

Key words

Academic stress, Body Mass Index, Serum Cortisol, Plasma Serotonin, ELISA

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INTRODUCTION

Stress in medical professionals starts before the beginning of professional life, and high levels of academic stress are frequently reported among undergraduates.[1,2] Several stressors confronted only by a medical student have been identified in a recently published review, and these include ethical conflicts, an increased load of work, exposure to sufferings and demise of human beings which often result in impairment of academic activities, academic dishonesty, disillusionment, cynicism, substance abuse and sometimes suicide.[3]

The terms "stress" was coined by Endocrinologist Hans Selye [2], and in 2011, Bruce McEwen and Jaap Koolhaas precisely defined it as an imbalance between controlling mechanisms [3] of individuals and environmental plus materialistic factors.[2,4] Stress increases the likelihood of severe systemic disorders such as central obesity [5], increased blood pressure(hypertension) [6], cardiovascular diseases, and metabolic syndrome [7] in addition to impaired function of the reproductive system of the body.[8]

It has been reported that compared to non-medical students, the overall prevalence of anxiety and depression, as well as the general psychological unrest, is more in medical students.[9] Studies from the Arab world have reported a similar level of anxiety and depression in medical students.[10] A number of consequences such as this excessive stress in medical students have been noted, such as negligence, self-medication, and increased commission of errors, decreased concentration, and attention at work.[10] The excessive amount of stress in medical training may lead to negative consequences such as diminished attention and focus in addition to cheating during exams. Medical education has, throughout the world, a very stressful curriculum, and the stress in medical school adversely affects the mental as

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well as physical health of students.[11] A number of factors play their role in the generation of stress such as the fear of failure in the examination and the associated peer pressure, the burden of parental expectations, inability to take time out for leisure activities, financial issues, relationship discords, and the ambitions of furthers studies in a medical career.[12]

The data regarding the level of stress in medical students in Pakistan are scarce and inadequate. Moreover, the research conducted so far does not show the biochemical parameters in Pakistani medical students. Cortisol is known as the main glucocorticoid hormone responsible for mediating the stress response.[13] Serotonin, also is known as 5-Hydroxytryptamine, is a monoamine neuromodulator that is found in Raphe Magnus nucleus of medulla. Serotonin helps in maintaining mood stabilization, pain control, emotional, cognitive, and motor activities like sleep and food intake.[14]
This study aims to measure the physical as well as biochemical parameters like cortisol and serotonin related to stress level in the body. Also to correlate the level of the stressors to influence on cortisol and serotonin concentrations with mood instability.

METHODOLOGY

Sample Size
The sample size was 50 Students from Final (5th) year MBBS students enrolled in Ayub Medical College who were healthy male/female volunteers aged 21 to 25 years with exclusion criterion of not taking any specific medications and smoking study .Sample size was calculated by WHO software.

Blood Sample collection
First Blood samples were collected on a normal working day considered as an unstressed state and then second blood samples on the day of examination were collected, taken as a stressed state. Blood samples were taken at the same time in the morning under strict aseptic techniques by, first of all, making participants comfortable and letting him sit in a chair in a relaxed upright position.[15]

Reseach Methodology
It was cross sectional (observational) study conducted in Ayub Medical College Abbottabad, Khyber Pukhtunkhwa, Pakistan. The subject selected were final year MBBS students of session 2018-19. Informed consent was taken for researcher and participant. Ethical approvals were obtained by the Graduate Studies Committee, Advanced Studies Research Board, and Ethical Committee of Khyber Medical University Peshawar. The ethical permission from the Principal of Ayub Medical College Abbottabad was also obtained to carry out this research work further.
All the experimental work related to participants and samples was carried out in laboratories of the Ayub medical college Abbottabad and Khyber Medical University Peshawar, between September 2019 and December 2019 and was done by researcher including Recruitment, Phlebotomy, biodata, lab work and data analysis. Specialized help was taken from the lab technician where necessary.

PHYSIOLOGICAL PARAMETERS:
Body mass index:
The weight of each volunteer was recorded barefoot with minimal possible clothing using a digital balance. The weight was recorded to 0.1kg. The scale was checked for zero error and adjusted accordingly.[16]
As per WHO guidelines fixed stadiometer was used to measure heights of the volunteers. Subjects were asked to stand barefoot with the back positioned against the scale with chin up, and arms relaxed on the respective lateral sides. They were instructed to take a deep breath, and the movable headboard was pressed on to the top of the head, with pressure enough to compress the hairs. The height was measured to the nearest 0.1cm.[13]
Body mass index (BMI) was calculated using the weight in Kilograms (Kg) divided by the square of the height in meters.
BMI = Weight (kg) / (Height in meter)

Measurements of Blood Pressure
Blood pressures were measured in supine posture using the sphygmomanometer CPE 2348 after the individual rested for 10 minutes. Most of the time, the right arm was used for checking the blood pressure, and in case of any ambiguity contra-lateral arm was used to confirm the readings are correct. [17]

Heart Rate
The heart rate of the volunteers was monitored manually by counting beats at the Radial artery by noting at Stopwatch.

Biochemical Parameters
Methods of blood sampling
Blood samples were taken at the same time in the morning under strict aseptic techniques by, first of all, making participants comfortable and letting him sit in a chair in a relaxed upright position. A disposable syringe of 10 ccs was used for taking 8ml of blood from the antecubital vein, hemostasis was secured by applying firm pressure with a swab, and first aid bandage was applied. Antiseptic protocols were followed.[18]
The blood samples were put on ice immediately after collection and centrifuged within 30 minutes of collection. A Universal centrifuge (800Z, 97101, centrifuge machine) spun the samples at 4°C and 1000 RPM for 15 minutes. Each sample of plasma and serum was divided into three aliquots and transferred into 0.5 ml Eppendorf tubes. After exact labeling, the plasma samples were stored at up to -80°C in an ultra-low temperature freezer for up to one month before analyses.[19]

Sample Analysis
All the samples, standards, and controls were thawed and brought to room temperature before analyses. The samples were analyzed for plasma serotonin (E1128Hu) from Bioassay Technology Laboratory and serum Cortisol, using ELISA kit Accu Bind) from Monobindinc. CA92630 USA. The samples were run in duplicates after dilution following the instructions of the manufacturer. A series of standards and controls were provided with each kit, and these were used to generate calibration plots

ELISA
The serum samples for cortisol and plasma samples for serotonin were analyzed through quantitative sandwich ELISA technique, which works on the principle of two antigenic determinants directed against two monoclonal antibodies. The first monoclonal antibody was fixed to the walls of microtitration well on the ELISA plate to which the antigen in the samples attaches. The second one was in a conjugate solution attached to the peroxidase enzyme. Unbound antibodies are washed away in the washing process, and a substrate solution was added to the wells. The substrate solution contains Tetramethylebenziden (TMB) and hydrogen peroxide, which produces color on reacting with the

enzyme-labeled antibodies. After incubation, the density of the color corresponds to the concentration of antigen in the sample [20]. In the end, the stop solution (1M H_2SO_4) was added, and the endpoint color density was noted with a spectrophotometer.

Cortisol Assay

Solutions were prepared and diluted and were brought to room temperature as per the description of the kit. The serum samples liquefy when kept at room temperature for 20 to 30 minutes. Standards were prepared in a series of dilutions. Exact procedures were followed, as mentioned in the guide book provided with the kit. .Serum cortisol was measured using the Cortisol Test System produced by Monobindinc (product code 3625-300).

Serotonin Assay

Serum serotonin is also measured by using the Human ST Elisa Kit (E1128 Hu), which uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to test the Human Serotonin (S.T.).

Statistical analysis of data

Statistical analysis was carried out using SPSS 22 statistical software. The normality of data was tested using Kolmogorov-Smirnov and Shapiro-Wilk tests. Mean, and the standard deviation was also checked for every variable. Summary statistics were calculated. Paired samples T-test were used to investigate the difference between means for stressed and unstressed states. A P-value of less than 0.05 was accepted as significant.

RESULTS

Results were obtained for all volunteers. Kolmogorov- Smirnov and Shapiro-Wilk tests were used to investigate the normality of the data sets. The data were normally distributed except for serum Cortisol concentration and plasma Serotonin concentration. The analyses were easier with the normal distribution of the data. Variables like Systolic Blood Pressure in a stressed state showed a normal distribution

Total data and characteristics of the population of study in stressed and unstressed states and comparison of males and females. Group statistics and paired samples T-test in the total population. A significant difference between stressed and unstressed participants was observed for Systolic B.P, Diastolic B.P, a p-value of < .01.

However no significant difference was observed between stressed and unstressed states for Age, Height, Weight, BMI (p= .94), Heart Rate (p= .149), Serotonin (p = .47), Perceived stress score (p= .011) and Cortisol (p=.72). Males were taller (p=0.001), heavier (p=0.35) and having higher BMI (p=0.001) and heart rate than females

The data of cortisol were checked for normality, which showed a non-normal distribution. In order to get meaningful results, the data were transformed by taking square roots and was rechecked for normality. The modified data were then used for the main analysis and revert back for discussing the results.

The mean Cortisol concentration for students in Unstressed condition was 6.6 µg/dl ± 4.2, while the mean Cortisol concentration for Stressed students was 7.0µg/dl ± 4.7. From the concentration of the means, it is evident that the difference is not significant, as observed through paired T-test (p-value = 0.72).

The Serotonin plasma concentration for all participants in the stressed and unstressed state was determined and checked for normality. The data was not normally distributed, as shown by Kolmogorov-Smirnov and Shapiro-Wilk tests, and can be readily appreciated with the naked eye.

The mean serotonin plasma concentration for students in stressed condition was 149.3 ±140.7while the mean serotonin plasma concentration for Unstressed students was 125.2±108.7. From the concentration of the means, it is evident that the difference is not highly significant as observed through paired T-test (p-value = .47).

DISCUSSION

This study evaluated Serum Cortisol and Plasma Serotonin concentration among medical students, which may be of importance to both medical students and college administration. The analyses undertaken in this study showed mean concentrations and values of all four parameters. We will discuss them one by one accordingly. The mean serum cortisol concentration in the stressed state was 7.0 µg/dl, and the unstressed state was 6.6µg/dl. The mean plasma serotonin concentration in the stressed state was 149ng/ml, and in an unstressed state, it was 125ng/ml.

Mean Serum Cortisol concentration in the stressed condition is 7µg/dl, and in an unstressed state, it is 6.6µg/dl when measured in 50 medical student volunteers, the results did not differ significantly (p=0.72). Our results are consistent in line with studies done by Bardi M, showing the same results (p=0.24) and by Meewisse in different reviews (p= 0.61).

Plasma serotonin level was not significantly different between students in stressed and unstressed states (p =.47), as observed in our study. Similar results (p=.68) were concluded by other studies as well. However, evidence from studies done by Vollmar in 2000, Haque in 2011, and Wang in 2013 suggests that plasma serotonin concentration changes significantly in the disturbed state (p < .001).Our study results investigated no rise in the stress hormone cortisol in a stressed state because stress in medical students influences and affects the psychological parameter more than biochemical parameter (11).

The study population of our study includes young adults (21yrs-25yrs) involving ten females and 40 males from the Final year MBBS class of Ayub Medical College Abbottabad. Results showed more physical impairments than biochemical disturbances. Parameters like Systolic B.P, Diastolic B.P with a p-value of <.01 showed a significant difference between stressed and unstressed participants. This study revealed high mood derangements with a psychologically and physiologically disturbed state of students. However other variables like Age, Height, Weight, BMI (p= .94), Heart Rate (p= .15), Serotonin (p = .47), and Cortisol (p=.72) showed no significant difference between stressed and unstressed conditions. In this study, male participants were taller (p = 0.001) and heavier (p=0.35) than females, and they showed more BMI values (p=0.001) and increased Heart rate.

A minimal amount of research has been conducted in Pakistani settings before, which measures both physical and biochemical parameters and their comparison in stressed and unstressed medical students. Our study is the first one to evaluate mood disturbances in medical students of Abbottabad by assessing any HPA axis dysregulation and changes in other essential variables like blood pressure and heart rate on the day of examination. The results of this study can be used as a reference source for further development of new techniques and methods for mood stabilization, grades improvement, and relaxation of students before any examination so that a more organized and student-friendly atmosphere can be arranged at institutes. Workshops and seminars can be organized to guide students on how to cope with mood imbalance in exam days.

	Stressed	Unstressed	p-value	Female	Male	p-value
	Mean ±S.D	Mean ± S.D		Mean ±S.D	Mean ± S.D	
Age (yrs)	24±1	24±1	-	24±1.1	24±1	0.3
SBP (mm of Hg)	126±12.2	113±10	**< .01**	134±9	124±12.3	**0.1**
DBP (mm of Hg)	92±9	90±9	**<.01**	97±8.3	92±9.2	0.28
Height (meters)	1.67±0.08	1.67±0.82	-	1.5±.03	1.6±.06	**<.01**
Weight (kg)	70.2±11.3	70.2±11.4	-	76±9.7	70.1±11.7	0.35
BMI(kg/m2)	24.69±3.2	24.63±3.2	.94	20.55±3	25±2.3	**<.01**
Heart Rate(b/min)	82±7.7	78±8.1	.14	78±2.5	84.2±8.1	**<.01**
Serotonin (ng /dl)	149.3±140.7	125.2±108.7	.47	83.7±46.1	165.6±152.2	0.25
Cortisol (ug /dl)	6.6±4.2	7.0±4.7	.72	2.6±1.2	2.4±1	0.59

Table-I: shows total data & characteristics of the population of the study, also shows stressed & unstressed values.

CONCLUSION

Results of our study highlighted that in the stressed state several physiological and biochemical changes occur which affect the efficiency of a student and also have an impact on personal life so It is time to disseminate the information to the administration of Medical institutes that stressful environment can precipitate mood disturbance and low productivity in medical students. This disoriented mood state can be improved by proper guidance and counseling sessions held regularly at colleges so that support to disturbed and stressed students can be provided to achieve good grades and remarkable academic scores.

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