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RESEARCH ARTICLE
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EFFECT OF ACADEMIC STRESS ON ENDOCRINE PROFILE AMONG MALE MBBS STUDENTS OF A MEDICAL COLLEGE

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Abstract:
Background: The effect of academic stress on the endocrine system is very complex but very few studies emphasize the association between the hormonal status with different grades of stress.
Aims: The study was done to establish the serum level of Cortisol, Prolactin, freeT4(fT4) and Thyroid Stimulating Hormone (TSH) as the biomarkers for academic stress.
Settings and Design: Cross-sectional descriptive study.
Methods and Material: Serum Cortisol, Prolactin, TSH and fT4 were measured in 123 male medical students during pre- examination, considered as a high stress period and post examination as a low stress period. Perceived Stress Scale (PSS) was provided to assess the level of stress among the participants at both time points.
Statistical analysis used: Differences in mean values, Correlation and ROC curve analysis done to formulate the diagnostic accuracy of each of the endocrinal parameters.
Results: All three parameters including Cortisol, Prolactin and fT4 except TSH were significantly increased with the increasing grade of stress. The ROC curve analysis illustrated a significant area under the curve (AUC) for Cortisol, Prolactin and fT4 with optimal threshold cut off value with high sensitivity and specificity.
Conclusions: These combined assays of serum Cortisol, Prolactin, and fT4 can be established as a diagnostic endocrine profile to assess the severity of academic stress This is probably one of the novel approaches to compare the diagnostic accuracy with the threshold cut off value of the individual parameter and the combination profile of the stress parameters (Prolactin, fT4 & Cortisol) with severity levels of stress.

Key-words: Academic stress, Prolactin, Cortisol, Thyroid Stimulating Hormone, Free T4

INTRODUCTION

Academic stress is widely recognized as a risk factor for depression in students, little is understood about the potential biochemical mechanisms underlying this association. Endocrine hormones and their effects as stressors in various stressful conditions are among the most prominent areas of study among researchers worldwide[1,2,3]. Medical students encounter various types of stressors during their studies which affects their learning abilities and academic performance adversely which often cumulates anxiety, depression, and even more vulnerable situations like suicidal tendencies

Numerous authors have documented that levels of endocrine hormones in serum are elevated as a response to various stress signals. Multiple studies have shown Cortisol to be associated with academic stress[3,4,5].When the hypothalamic-pituitary-adrenocortical (HPA) axis is activated by stress, it stimulates the hypothalamus to secrete an adrenocorticotropic hormone-releasing hormone (ACTH-RH), which activates the anterior pituitary to secrete Adrenocorticotropic Hormone (ACTH). The adrenal cortex is stimulated by ACTH to release cortisol. Very few past researches focused on Prolactin, another hormone released from the anterior pituitary, and Thyroid hormones, fT4 and TSH on their effects on stress levels[5,6,7,8]. The extent to which academic stress is linked to endocrine stress that responds to hormonal status remains unclear in light of several contemporary studies. The study was designed to find out the relationship between academic stress levels subjectively studied by the PSS stress questionnaire with the status of endocrine hormones (Cortisol, Prolactin, fT4, and TSH) among the medical undergraduates (MBBS) students, the most vulnerable segment of the Indian population. The study aims to alleviate the problem of academic stress among medical students, by interventions directed towards management of academic stress therapeutically.

AIM OF THE STUDY

The study was done to establish the serum level of Cortisol, Prolactin, freeT4(fT4) and Thyroid Stimulating Hormone (TSH) as the biomarkers for academic stress.

OBJECTIVES

1. To assess stress level using Perceived Stress Scale (PSS)
2. To estimate serum hormones & their inter-relationship with stress level.

SUBJECTS AND METHODS

This was a cross-sectional descriptive study performed at a medical college in Kolkata, India, in the Department of Biochemistry. The study was approved by the institutional ethics committee, All the procedures in this study involving human participants were in accordance with the ethical standards of the institutional and national research committees and the Declaration of Helsinki (October 2013). Informed consent was obtained from all the study subjects.

Selection of participants

A simple random sampling of 123 students out of a total of 160 volunteer-based participants was recruited from first-year male medical students. There was no predetermined sample size. Exclusion criteria were a history of acute illness, previous known medical condition, or any psychopathological problem.

Experimental design

Study performed in two time periods 1) Pre-examination period: 7 days before examination **(Group-1)**. Post-examination period: two weeks after the examination **(Group-2)**. The Pre-examination period was considered as the high-stress setting and the second period was considered a minimal-stress setting. Stress levels perceived through questionnaires and blood for analysis of biochemical parameters were done at both time points. Blood samples and questionnaires were coded for the maintenance of anonymity.

Stress questionnaire

A self-report validated Perceived Stress Scale (PSS) was provided to assess the level of stress among the participants at both time points. Individual scores on PSS ranged from 0-40 and the questionnaire was designed to segregate participants into mild, moderate, and severe grades of stress.

Biochemical analysis

Fasting Blood samples from all participants were collected at both time points between 8:00 a.m. to 9:00 a.m. Samples collected in vacutainers were subjected to centrifuge for serum separation. All the samples were assayed for Cortisol and Prolactin by chemiluminescent immunoassay (ADVIA Centaur) and fT4 and TSH by Electrochemiluminescence (COBAS) at the Central Laboratory of medical college.

Statistical analysis:

All data were collected, compiled, and subjected to suitable statistical analysis using appropriate methods with SPSS, version 20 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). Quantitative data were evaluated for normal distribution using the Kolmogorov-Smirnov test. Data were presented as mean ± standard deviation for parametric data (Prolactin) and median (interquartile range) for non-parametric data (Cortisol, f T4 and TSH) or number and percentage as appropriate.

Differences in mean values between the groups were determined using a One-way analysis of variance (ANOVA) for parametric data (Prolactin) and Kruskal Wallis for non-parametric data (Cortisol, f T4 and TSH). Multiple comparisons between the differences in means were performed using the appropriate Posthoc test. Pearson correlation coefficients were used to test the correlation between Prolactin levels with the different stress groups. Spearman's Rho correlation between Cortisol, fT4 and TSH with grades of stress. Paired t-test of Cortisol, Prolactin, fT4 and TSH was done between Group-1 study population (Pre-examination) and Group-2 study population (Post-examination) and the ROC curve analysis done to formulate cut off thresholds of each parameter with stress severity and establishing the diagnostic accuracy of each of the parameters. Results were considered statistically significant for p-values<0.05.

RESULTS

One hundred twenty-three healthy male MBBS students who were graded according to their level of stress using PSS questionnaire. Out of 123 subjects,40 students had mild grade, 52 students had moderate grade and 31 students had severe grade of stress. Table 1 shows the mean level of Cortisol, Prolactin, fT4 and TSH in the Group-1 study population (Pre-examination) according to the grade of stress. All the parameters except TSH were significantly increased with the increase of the grade of stress.

Table 1: Prolactin (ng/ml), Cortisol (µg/dl), fT4 (ng/dl) and TSH (µIU/ml) in the Group-1 study population (Pre-examination)

Variable	Mild Stress (N=40)	Moderate Stress (N=52)	Severe Stress (N=31)	p-Value
Prolactin (ng/ml)	6.62 ± 0.62*	8.58 ±0.47*	13.63±0.47*	.001**
Cortisol (µg/dl)	15.21(6.64) ǂ	19.54(5.55) ǂ	24.65(2.02) ǂ	.001 €
fT4 (ng/dl)	1.07(0.38) ǂ	1.77(0.92) ǂ	2.84(1.05) ǂ	.001 €
TSH (µIU/ml)	2.83(2.19) ǂ	2.81(2) ǂ	2.8(2.92) ǂ	0.950 €

*Mean ± Standard error; ǂMedian(Interquartile Range).
**p-Value (ANOVA); €p-Value(Kruskal Wallis Test)

Table-2 shows the correlation of Cortisol, Prolactin, fT4 and TSH in the Group-1 study population (Pre-examination). During the Pre examination period significant correlation was found between Cortisol, Prolactin, fT4.Significant increase in the proportion of variance in the level of Cortisol, Prolactin and fT4 with the grade of stress.

During the Post examination period no such correlation was found between the PSS Stress scores and the level of Cortisol, Prolactin and fT4.Correlation between TSH and grade of stress was insignificant in both pre and post examination period.

Table 2: Correlation coefficients of Prolactin (ng/ml), Cortisol (µg/dl), fT4 (ng/dl) and TSH (µIU/ml) in the Group-1 study population (Pre-examination)

Variable	Mild Stress (N=40)	Moderate Stress (N=52)	Severe Stress (N=31)
Prolactin‡ (ng/ml)	**0.238****	**0.520***	**0.654***
Cortisol € (µg/dl)	**0.505***	**0.595***	**0.860***
fT4 € (ng/dl)	**0.508***	**0.554***	**0.624***
TSH € (µIU/ml)	**0.141****	**0.129****	**0.018****

*p<0.01 ;**p>0.05
‡Pearson Correlation €Spearman's Rho Correlation

Table-3 shows the Paired t-test of Cortisol, Prolactin and fT4 between Group-1 study population (Pre-examination) and Group-2 study population (Post-examination). There is significant decrease in the level of Cortisol, Prolactin and fT4 in the Post examination period (Group-2) in comparison to the Pre examination period (Group-1). No significant changes in the level of TSH could be observed.

Table-3: Paired t-test of : Prolactin (ng/ml), Cortisol (µg/dl), fT4 (ng/dl) and TSH (µIU/ml) between Group-1 study population (Pre-examination) and Group-2 study population (Post-examination)

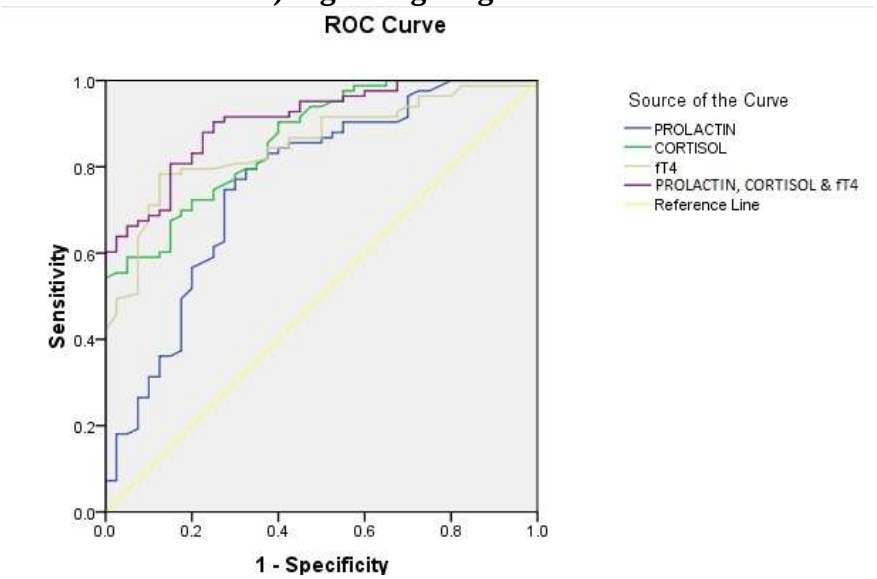
Variable	Pre-examination period	Post Examination period	Paired Difference		t value	pValue
			Mean	Std.Error		
Prolactin (ng/ml)	9.22±0.39*	6.90±0.27*	2.313	0.46	4.96	<0.01
Cortisol (µg/dl)	19.42±0.44*	9.36±0.24*	1.004	0.47	21.11	<0.01
fT4 (ng/dl)	1.74±0.069*	1.31±0.04*	0.429	0.058	7.28	<0.01
TSH (µIU/ml)	2.68±0.12*	2.64±0.11*	0.411	0.07	0.56	0.577

*Mean±Standard Error

The ROC curves were analysed and were plotted as shown in Figure-1 for the diagnostic value of the serum Cortisol, Prolactin and fT4 in moderate and severe grades of stress in comparison to mild grade of stress. The analysis of ROC curve illustrated a significant area under the curve (AUC) for Cortisol, Prolactin and fT4 with optimal threshold cutoff value with high sensitivity and specificity. AUC for TSH was found to be insignificant.

AUC for the combination of all the three parameters i.e. Prolactin, Cortisol and f T4 in combination as stress indicator was significantly high which provides a highly positive diagnostic accuracy for the grade of stress in the study population.

Figure 1: Receiver operating characteristic plots demonstrating discriminatory ability of Cortisol (µg/dl), Prolactin (ng/ml), fT4 (ng/dl) in the Group-1 study population (Pre-examination) regarding diagnostic value of stress



VARIABLE	AUC	CUT OFF	SENSITIVITY	SPECIFICITY
Cortisol (µg/dl)	0.865	17.78	78.3%	70%
Prolactin (ng/ml)	0.762	6.81	80.7%	65%
fT4 (ng/dl)	0.854	1.23	80.7%	70%
Combination of Prolactin, Cortisol &FT4	0.904	-	-	-

DISCUSSION

Academic stress continues to be a devastating issue affecting a student's mental health and general well-being[9]. Researches have revealed the largest source of stress for medical students was related to the exhaustive medical curriculum creating a feeling of academic disappointment[10,11,12].Medical students who experience academic stress may have mental instability, poor judgment, and absenteeism from class, all of which can negatively impact their overall academic performance[13,14,15].The present study was done to establish the serum level of Cortisol, Prolactin, f T4 and TSH as the biomarkers for academic stress.

According to the study, medical students' levels of cortisol, prolactin, and fT4 significantly increased when their stress levels increased throughout the pre-examination phase. The degree of academic stress was significantly correlated with the levels of cortisol, prolactin, and fT4 in the serum, as evidenced by correlation studies. These changes in the endocrine profile were minimal with mild stress and maximal with severe stress. Additionally, a lower PSS score and a subsequent significant decrease in cortisol, prolactin, and ft4 levels indicated that stress levels had decreased throughout the post-examination interval.

This present study corroborated with the study of Laila Y. Al-Ayadhi et al (2004), which showed that both Cortisol and NPY plasma levels increased during the academic stress time as compared to the baseline. Both these studies indicated that the two systems, that is the hypothalamus-autonomous nervous system (HANS) and the hypothalamic–pituitary–adrenal axis (HPA), both responded in similar ways. Neuropeptide Y(NPY) has been coupled to other stress parameters such as norepinephrine, in response to sympathetic activation[3].Furthermore, the elevated plasma level of ACTH, which is released from the anterior pituitary and acts on the adrenal cortex to release Cortisol, further proves the involvement of the hypothalamus-pituitary-adrenal axis in this type of stress[16].

The findings of Anna-Karin Lennartsson et al (2011) were also in accordance with the present study stating the significant increase in elevated Prolactin levels along with significantly increased plasma ACTH and serum Cortisol, in response to the stressor. The magnitude of the Prolactin response was significantly related to the magnitude of the response of the hypothalamic–pituitary–adrenal (HPA)

axis and, to some extent, the cardiovascular responses, indicating that individual differences in Prolactin response in healthy men and women are dependent on the general physiological stress activation[17]. The findings of Bako Ibrahim Gaya et al. (2014), who suggested that elevated prolactin levels might be a helpful neuro-endocrine link to academic stress, were supported by the current investigation, which found elevated prolactin levels with an elevated stress response[18].

The evidence from studies on patients with Cushing syndrome and hyperthyroidism that changes in cortisol levels during stress can impact the thyroid hormone level and that T3, T4, and stress had a significant relationship. supported the study's findings of a significant increase in free T4 levels and non-significant changes in TSH levels. Conversely, Anjum A et al[19]. observed no significant difference in T4 and TSH in the time interval between the pre and post examinations, whereas other researchers discovered a substantial positive correlation between stressful conditions and subclinical hypothyroidism in their study populations[20]

The observations of the present study along with the previously reported supportive studies cumulatively substantiates the hypothesis of a novel pathway through which elevated levels of Cortisol, Prolactin and f T4 has a synergistic effect on severity of academic stress. Thus, the endocrine profile constituting of serum Cortisol, Prolactin, and f T4 can be established as stress biomarkers specifically academic stress among male medical students which can further be the harbinger in the development of therapeutics targeted in combating the severity of level of stress, particularly academic stress.

CONCLUSION:

The current study concludes that there is a significant increase in serum cortisol, prolactin, and fT4 with the severity of academic stress. Therefore, the combined assays of serum cortisol, prolactin, and fT4 can be established as a diagnostic endocrine profile to assess the severity of academic stress. This is probably one of the novel approaches to compare the diagnostic accuracy of the combination profile of the stress parameters (Prolactin, fT4 & Cortisol) with severity levels of stress (by PSS questionnaire) among medical students considering examination as the stress stimulant.

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AUTHOR'S CONTRIBUTIONS:

Concepts & Design: Papia Sen, Soumika Biswas and Sangita Biswas
Literature search & Data acquisition: Papia Sen, Sangita Biswas, Moinak Dutta
Statistical analysis: Papia Sen
Manuscript preparation: Papia Sen, Soumika Biswas and Sangita Biswas
Manuscript editing &review: Papia Sen.

CONFLICT OF INTEREST: NIL

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