

Serum Cortisol, Progesterone and Total Antioxidant Status of Students Pre-and Post-Examination

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Abstract

Background: Stress is defined as a mental or emotional strain resulting from very demanding circumstances. In recent years, traumatic episode and poor results following school examination have made stress an issue of major concern in academics. This study aimed to investigate examination induced stress among apparently healthy University students in relation to some biochemical parameters and antioxidants status pre-and post-examination.

Materials and Methods: This is a case controlled study involving 40 examinees as test group and 40 non-examinees as control group. Serum concentrations of cortisol and progesterone were estimated by ELISA method, and total antioxidants status (TAS) was quantified by colorimetric method. Data obtained were subjected to statistical analysis using SPSS software application (version 16.0) and the results expressed as mean \pm standard deviation.

Results: The results showed that cortisol level was significantly decreased in pre (34.84 \pm 8.50) examination than in post (21.18 \pm 14.53) examination while progesterone level was significantly increased from 0.42 \pm 0.32 to 0.92 \pm 0.96 ($p < 0.05$) and TAS increased from 0.97 \pm 0.05 to 1.02 \pm 0.32 ($p > 0.05$). The mean serum cortisol in subjects with outstanding academic activity was not statistically significant when compared to subjects without outstanding academic activity ($p > 0.05$).

Conclusion: the differences in the biochemical parameters observed in this study pre and post examination suggest that examination has the tendency to induce stress which could culminate to oxidative damage in chronic cases.

Keywords: Stress, examination, students, University

Introduction

Stress is a constellation of event which is composed of a stimulus called stressor that precipitates a reaction in the brain (stress perception) which subsequently activates a physiologic (fight/flight) system in the body which is called stress response¹. Examination is a formal and informal assessment of learning and tests of students' ability to perform specific tasks². It is designed to test students' performance giving an indication of

their ability to cope under pressure in a fixed period. This is a constant feature in Nigerian universities. Stress due to examination is a feeling that is created when a student is faced with examinations on accumulated courses over a period pre-determined by the examination time table and this usually occurs within one to four weeks and mostly at the end of the semester.

There are many causes of stress and these range from minors such as noise, thinking and day to day activities³ to the major ones such as too many assignments,

competition within a chosen field, failure, financial worries about school, inadequate resources to perform academic work, overcrowded hall, poor relationship with other students or lecturers, inadequate classroom, bad conditions of university hostels, long walk to school, etc^{4, 5, 6}.

Other sources of stress include the ever increasing rate of student enrolment without accompanying support facilities, lack of adequate counseling programmes⁷, poor welfare programmes (power failure, absence of pipe borne water for the students, non-provision of Iran spoliation system, etc), poor time management and study habit skills, poorly planned orientation and character development programmes and poor health programme⁸.

Stress is generally classified into acute and chronic stress depending on the duration of exposure⁹. Acute stress is the stress that last for a period of minutes to hour and chronic stress persists for several hours to weeks or months. However, stress is also classified on the basis of stressors as physical/environmental, emotional/psychological¹², and mental stress. Examination can induce both menial and psychological stress¹³. However, in Nigerian universities and many other developing countries especially in Africa students experience all these three types of stress; Physical stress from poor infrastructures in the schools, increased anti-social vices, unconducive learning environment. Emotional stress from a broken home, non-caring father or mother, death of a loved one etc. menial stress from accumulated class work due to incessant industrial break, courses that are supposed to be thought in fifteen weeks are thought in a less time.

Stress produces definable mental and physiological reaction in the body¹⁴. Mild stress is beneficial in cognitive last and performance but persistently high stress lead to neuropsychiatric illness like anxiety and depression. Examination acts as a stressor and activate hypothalamic pituitary adrenal axis causing increase in cortisol level¹⁵. When stress is perceived negatively or become excess, it can affect both health and academic performance⁶. Mark et al.¹⁴ and Serkan et al.¹⁵ in two separate studies reported that stress induce elevation in the plasma cortisol level. The stress levels are different among university students and also different between male and female¹⁶. Medical education is perceived as being stressful with negative effects on student's menial health. However, few studies have addressed the influence of

gender on stress in Medical students. Research findings suggested that male students feel stronger stress from family factor than female ones¹⁷. Among other effects, the human Glucocorticoid cortisol is able to permeate the blood-brain barrier, where it influences the activity of the central nervous system by activating central corticosteroid receptors¹⁸.

The production of Reactive Oxygen Species (ROS) and reactive nitrogen species (RNS) is the major effect of this immune-endocrine interaction in stressful situations. The ROS damages vital biological system by forming oxidative modified biomolecule (protein, lipids, and nucleic acids) through peroxidation of lipids^{19, 20}. This oxidation distorts the organization and the relationship in molecules and this forces living organism to develop antioxidant defense system.

Materials and Methods

This study recruited 80 students of Nnamdi Azikiwe University (NAU), Nnewi Campus, between the ages of 21-26 years. Forty of these students were writing examinations and they served as test group while control group (n=40) were also recruited randomly from among students not writing any examination. Questionnaire was administered to all the participants in order to obtain information concerning their age, sex-lifestyle, duration of study, level of study and some other information as regards difficulty encountered in course of study. Based on the information gathered from the questionnaire, the participants were further sub-grouped into two groups.

(i) Group one (Outstanding academic activity): These are the participants who were repeating class, had failed courses to write, reduction on the amount of time spent on studies, accomplished less than they would, had difficulty in understanding and had examination on a regular basis, (n-24)

(ii) Group two (No outstanding academic activity): These are the participants who were not repeating class, had no failed courses to write, had no reduction on the amount time spent on studies, accomplished more than they would, had no difficulty in understanding and had examination on a regular basis (n=16).

Inclusive and Exclusive Criteria

Only students who are writing examinations were

recruited as test group. Those on hormone therapies, psychotic drugs or with diagnosed endocrine disorder were excluded from the study. Also those who are addicted to substances such as alcohol, cigarettes, and females on their menstrual period excluded.

Sample Collection

Venous blood (5ml) was collected from the test group and the control group between 6:30am to 8:30am. After the examination, another sample was collected from the test group between 11:00 am to noon. The 5ml was dispensed into a plain container was allowed to clot and retract and separated using a centrifuge. The samples were stored frozen (-20°C) until the time of analysis. The samples were analyzed for cortisol, progesterone and total antioxidant.

Methods

Cortisol and progesterone estimation were done using Enzyme Linked Immunosorbent Assay by Foster and Dunn,²¹ and Voller et al.²² respectively.

Total Antioxidant status was estimated using colorimetric method by Koracevic et al²³.

Results

The effect of examination on serum concentrations of cortisol, progesterone and TAS

The mean serum cortisol and progesterone levels showed an inverse pattern, cortisol was highest in the pre examination group and lowest in the control group while progesterone was lowest in the pre examination group and highest in the control group and when compared between the groups was statistically significant ($p < 0.05$). Also, the mean serum level of TAS was significantly higher in control group and lower in pre examination group and when compared between the groups ($p < 0.05$) (Table 1).

Sex distribution of the effect of examination on serum concentrations of cortisol, progesterone, and total antioxidants status

The mean serum cortisol was highest in the pre examination group and lowest in post examination group in both male and female participants and when compared between the groups was statistically significant ($p < 0.05$). The mean serum total antioxidant status was low in both male and female in the pre examination groups

and highest in both male and females in control groups, the serum TAS when compared between the groups was statistically significant ($p < 0.05$) (Table 2).

The effect of Out-standing academic activity and No out-standing activity on the biochemical parameters pre-examination

The mean serum cortisol was highest in participants with out-standing academic activity compared to those without outstanding academic activity but was not statistically significant ($p > 0.05$). Also, the mean serum progesterone was lowest in participants with out-standing academic activity compared to subjects without out-standing academic activity but when compared between the groups was not statistically significant ($p > 0.05$). Mean serum TAS in the two groups were almost the same ($p > 0.05$) (Table 3).

Correlation of cortisol in pre- and post-examination

A weak negative insignificant correlation in cortisol was shown in pre- and post-examination while serum concentrations of progesterone and total antioxidant status showed a weak positive insignificant correlation ($p > 0.05$).

Correlation of cortisol pre- and post-examination in both the males and female students

A weak negative insignificant correlation in cortisol was observed in pre- and post-examination in both the males and females participants ($P > 0.05$), while the pre- and post-examination total antioxidant status in males and females show weak positive and yet insignificant correlation ($P > 0.05$). The pre- and post-examination serum progesterone in the males was also found to be insignificantly correlated ($P > 0.05$). The correlation analysis carried out between the post-examination cortisol and post-examination antioxidant status in the males yielded a negative insignificant correlation ($P > 0.05$). The serum concentration of progesterone when correlated with the total antioxidant status post examination showed a weakly positive insignificant correlation ($P > 0.05$). The mean serum concentrations of pre examination cortisol and pre-examination progesterone showed a strong negative correlation ($p < 0.05$). There were no significant correlation in other parameters compared ($p > 0.05$).

Table 1: Biochemical parameters in Pre and Post examination

Group	Cortisol(ng/ml) n=40	Prog (ng/ml) n=24	TAS (mmol/l) n=40
Control (A)	20.40±9.02	1.28±0.70	1.65±0.12
Before Exam (B)	34.84±8.50	0.42±0.32	0.97±0.05
After Exam (C)	21.18±14.53	0.92±0.96	1.02±0.32
F- value	21.152	7.218	405.281
P- value	< 0.001*	0.002*	<0.001*
A/B	< 0.001*	<0.001*	<0.001*
A/C	0.756	0.117	<0.001*
B/C	< 0.001*	0.025*	0.053

Prog-progesterone, TAS- total antioxidant status,* the mean difference is

significant at 0.05 level.

Table 2: Serum cortisol progesterone and TAS before and after examination

Groups	n	Cortisol (ng/ml)	Prog (ng/ml)	TAS (mmol/l)
Control Male (A)	24	18.67±8.45	1.28±0.70	1.63±0.11
Control Female (B)	16	22.63±9.48		1.68±0.14
Pre Exam Male (C)	24	37.70±7.59	0.42±0.32	0.97±0.06
Pre Exam Female (D)	16	31.13±8.35		0.98±0.03
Post Exam Male (E)	24	19.49±14.01	0.92±0.96	1.00±0.03
Post Exam Female (F)	16	23.35±15.30		1.06±0.16
F- value		9.879	7.218	164.283
P-value		<0.001*	0.002	< 0.001*
A/B		0.262		0.263
A/C		<0.001*	<0.001	<0.001*

A/E	0.804	0.117	<0.001*
B/D	0.025*		<0.00*
B/F	0.848		<0.00*
C/D	0.064		0.821
E/F	0.274		0.122
C/E		0.025*	

Prog-progesterone, TAS- total antioxidant status, * the mean difference

is significant at 0.05.

Table 3. Biochemical Parameters of Students with/without Out-standing Academic Activity before Examination

Parameter	Outstanding Academic activity n=24	No outstanding academic activity n=16	T -value	P- value
Cort (ng/ml)	39.54±6.70	34.67±8.06	1.96	0.057
Prog (ng/ml)	0.39±0.20	0.52±0.42	-0.920	0.368
TAS(mmol/l)	0.98±0.03	0.96±0.08	1.20	0.236

Cort- cortisol, Prog -progesterone, TAS-total antioxidant status.

Table 4. Correlation coefficient between cortisol, progesterone and total antioxidant status

Parameters	n	r-value	p-value
Pre cortisol Vs Post cortisol	40	-0.051	0.753
Pre prog vs Post prog	24	0.053	0.807
Pre TAS vs Post TAS	40	0.159	0.326

TAS = total antioxidants status, - = negative correlation

Table 5: correlation coefficient between sex distributions

Parameter	Male (n= 24)		Female (n= 16)	
	r-value	P-value	r-value	P-value
Pre cortisol vs Post cort	-0.139	0.517	-0.123	0.650
Pre prog vs post prog	-0.038	-0.862	-	-
Pre TAS vs post TAS	0.100	0.643	0.080	0.768
Post cort vs post TAS	-0.253	0.233	-0.281	0.298
Post prog vs post TAS	0.030	0.889	-	-
Pre cort vs pre prog	-0.468	0.021*	-	-
Pre cort vs post prog	0.223	0.294	-	-
Pre cort vs pre TAS	0.199	0.351	-	-
Pre cort vs post TAS	-0.245	0.248	-0.274	0.305

P value is significant of $P < 0.05$, *-significant, - =negative correlation, n=40,

Prog -progesterone, TAS-total antioxidant status, cort-cortisol.

Discussion

Stress is a mental or emotional strain resulting from a very demanding circumstance. In recent years, traumatic episode and poor results following school examinations have made stress an issue of major concern in academic. This study evaluated the effects of examination on students in relation to serum concentrations of cortisol, progesterone, and total antioxidants status. In the findings of this study, pre-examination serum concentration of cortisol was found to be higher in examinees when compared to the non-examinees. This reveals the stress which accrued during the preparations for the examination. The significant decline in cortisol level post-examination when compared to the pre-examination cortisol level suggests a relief in mental stress. This further buttresses the fact that examination can induce stress. It has been reported that stress influences the activity of the hypothalamic pituitary adrenal axis and consequently increase the serum cortisol level²⁴. Cortisol is increased in stress and it is a mediator of stress response

because adrenalectomised animals and patients with Addison syndrome lure poorly when stressed²⁵. Vaidya and Mulgaonkar,²⁶ also found increased level of stress and anxiety among first year medical students and they found academic pressure to be responsible for this. Several research works have shown increased levels of cortisol secretion in response to academic stressors or laboratory stressors^{27, 28}. However, Patricia et al,²⁹ in their study observed lower levels of cortisol during examination stress while Larson et al,³⁰ reported no difference in plasma cortisol before, during and after examination. Cortisol affects and enables the coordination of the brain and body functions involved in coping with a stressor³¹. Lecker et al,³² suggested in their study that elevated levels of cortisol if prolonged can lead to proteolysis and muscle wasting. When sex distribution was considered, there was no statistical difference observed. Earlier work published by Verma et al,³³ on the effect of gender difference on cortisol response and Ennis et al,³⁴ reported that while cortisol increased in men anticipating an examination, it

decreased in women. However, the correlation analysis carried out between the pre- and post- examination cortisol level was found to be insignificant both in male and female examinees. The serum cortisol level was found increased in students with out-standing academic activities when compared with students without outstanding academic activities, but insignificant. Imam et al,³⁵ reported increased serum cortisol level with an increase in the intensity of perceived examination stress. A negative correlation was observed between serum concentrations of cortisol pre-examination and progesterone pre-examination. This could be adduced that the decrease in progesterone level leads to increase in cortisol level as a result of examination induced stress. During stress, progesterone secretion is inhibited to conserve energy for the imminent stress response³⁶.

A significant decrease in serum progesterone level was observed in the examinees pre-examination when compared with the control subjects. "This could be linked to stress which developed in the course of the preparation for the examination. The significant increase in progesterone level post examination level further fingers stress as a possible factor that may have lead to the decrease in progesterone level and suggest that progesterone may play a crucial role in regulation of the activities of stress system. Evidences from both human and animal studies suggested that progesterone modulates response to stress. In laboratory animals, plasma levels of progesterone are elevated alter acute stressors³⁷. In human, stress induces increase in progesterone and allopregnenolone^{38,39}. Onyenekwe et al,³⁶ reported a similar finding and further suggested that the decrease in pre-examination progesterone which returns after examination may be to restore homeostasis or due to gradual setting-in of pregnenolone steal phenomena or even due the fact that cortisol and progesterone compete for a common receptor in the cells resulting to impairment in the progesterone activity. The adrenal secretion of progesterone is increased in response to stress, and progesterone secretion by the adrenal glands can become comparable to that of the ovaries in stressed rats⁴⁰. Studies have shown evidence of neuroprogesterone synthesis by neurons and Schwaan cells in the peripheral nervous system⁴¹. The role of progesterone in attenuating the stressed induced increases in ACTH has been reported⁴². According to Sanchez et al,⁴³ there is increase in enzyme that converts progesterone to allopregnanolone. In another study, Crowley and Girdler,⁴⁴ reported the role of

progesterone and its metabolites in restoration of the activity of the hypothalamic pituitary adrenal axis after a stressor. Our findings support the idea that progesterone and possibly its metabolites such as allopregnanolone and pregnanolone play a role in recovery from stress⁴⁴. Stress response is crucial for survival and must be readily activated, but must also be limited in duration to avoid harmful effects⁴⁵. The role of neuroactive steroids in the regulation of the activity of stress systems has been reported⁴⁴. Childs and colleagues⁴² reported that progesterone (50mg and 100mg) attenuated stress induced increase in alertness, arousal and negative mood. Progesterone attenuated stress-induced increases in anger and frustration and promoted recovery from negative mood changes after stress. Lovick⁴⁶ reported that progesterone decreases anxiety-like behaviors. When the pre-examination progesterone level was compared to post-examination progesterone level, an insignificant correlation was observed. The serum concentration of progesterone in the subjects with outstanding academic activity was found to be lower than that found in subjects without out-standing academic activity but not significant. The latter, could be connected to the effect of stress on mental performance. Noreika et al,⁴⁷ found that individual differences in levels of progesterone and its metabolites were correlated with subjective measures and performance in cognitive task; these results suggest that progesterone may preferentially play a role in psychological or subjective responses to stress in humans.

This study showed a significant decrease in the total antioxidant level in the pre examination group. And, when gender distribution was considered, there was no significant difference observed. This finding of decreased total antioxidant status is an indication that examination can induce oxidative stress. Similarly our data of decreasing antioxidant level before examination is in accord with results published by Sivonova et al,⁴⁸ Irie et al^{49, 50} reported an increase in oxidative damage to DNA during the presumed psychological stress before examination. Legard et al,⁵¹ studied an influence of different life style factors on this lest system which measured the resistance of red blood cells to an oxidative challenge, their study showed that psychological stress is a major factor influencing antioxidant status. A similar conclusion was reached by Chalmer

et al.⁵² However; decreased antioxidant capability may be caused by the depletion of antioxidant as a result of oxidation. During psychological stress, increasing circulating level of catecholamine which is a source of reactive oxygen species may play a role in decreasing antioxidants level^{48, 53}. Free radical mediated damage has been incriminated in aging and in the genesis of many chronic diseases such as cancer, cardiovascular diseases, diabetes, and inflammatory diseases⁵⁴. Young and Woodside,⁵⁵ and Xu,⁵⁶ suggested that the classical antioxidant molecule such as vitamins E, C, and A, flavonoids and other phenolic compounds contained in fruits and vegetables play important roles in enhancing plasma antioxidant capacity of humans

Conclusion

The increase in serum concentrations of cortisol and decrease in serum progesterone highlights the tendency of examination to induce stress. The decrease in antioxidants level in students is an indication of oxidative stress. With constant effect resulting from examination conditions, oxidative stress could lead to oxidative damage if not checked. It is pertinent to reduce examination induce stress by increasing the interval between examinations. This will help to prevent eases of oxidative stress culminating the oxidative damage. Further longitudinal studies are required to determine the effect of examination induced oxidative stress in University students.

Ethical Clearance : Ethical approval was obtained from Ethics Committee of Faculty of Health Sciences and Technology, Nnamdi Azikiwe University Nnewi Campus. The participants gave informed consents and no monetary inducement was made to the participant.

Conflict of Interest – NIL

Source of Funding- Self

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