



**International Journal of TROPICAL DISEASE
& Health**
4(1): 62-69, 2014



SCIENCEDOMAIN *international*
www.sciencedomain.org

**Effect of Pre and Post Academic Examination
Stress on Serum Level of Cortisol and
Progesterone Circulation amongst Students of
Nnamdi Azikiwe University Nnewi Campus
Anambra State, Nigeria**

**Onyenekwe Charles Chinedum¹, Ezeani Michael Chukwudi^{2*},
A. Udeogu Ndidiamaka¹, C. Anyiam Daniel³, U. Meludu Samuel⁴
and Nnadozie Okwudiri⁵**

¹Department of Medical Laboratory Science, Nnamdi Azikiwe University Nnewi Campus,
Nigeria.

²Immunology Department, Nnamdi Azikiwe University Nnewi, Nigeria.

³Department of Histopathology Nnamdi Azikiwe University Nnewi Campus, Nigeria.

⁴Department of Human Biochemistry, Nnamdi Azikiwe University Nnewi, Nigeria.

⁵Chemical Pathology Unit Nnamdi Azikiwe University Teaching Hospital Nnewi, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors OCC and UMS designed the study, performed the statistical analysis. Authors EMC and NO conducted and managed the Laboratory analysis, author EMC wrote the manuscript. Author AUN conducted Laboratory analysis, managed the literature search. Author CAD wrote the protocol, conducted laboratory analysis. All authors read and approved the final manuscript.

Original Research Article

Received 14th February 2013

Accepted 23rd April 2013

Published 24th October 2013

ABSTRACT

Stress has become an important issue in academic circle as well as in our society. Academic examination stress was considered an important factor to increased circulation of cortisol and progesterone amongst students. Therefore this study was designed to determine cortisol and progesterone levels in apparently healthy students (n=30) during a

*Corresponding author: Email: mikezeani@yahoo.com;

day examination and those who have no examination served as control subjects (n=30). Serum levels of cortisol (ng/ml) and progesterone (ng/ml) were determined before examination in the test group and Control group and after examination in the test group alone. Serum level of albumin (g/l) was also determined in all the subjects. The result obtained showed that serum levels of cortisol, was significantly higher in pre-examination when compared with the post examination ($P<0.001$) while serum level of progesterone was significantly lower in pre-examination when compared with the post-examination ($P<0.01$). The mean level of cortisol was significantly higher in pre-examination subjects when compared with the control subjects ($P<0.01$) while the mean level of progesterone was significantly lower in pre-examination subjects when compared with the control subjects ($P<0.01$). The result also revealed that the mean level of progesterone was significantly higher in control subjects when compared with the post-examination ($P<0.01$), while there were no significant difference in the mean levels of both post-examination cortisol and albumin in the test subjects compared with the control subjects (>0.1). The results showed there were significant levels of negative correlation between pre-progesterone and pre-cortisol ($r=-0.399$; $P<0.05$) and pre-progesterone versus post-progesterone ($r=0.419$; $P<0.05$). This study indicates that serum level of cortisol increases significantly while serum progesterone level decreases significantly before the examination. This may have implication for clustered examinations within many days.

Keywords: Examination stress; cortisol, progesterone; students; Nigeria.

1. INTRODUCTION

Although everyday academic stress may not show immediate health effects, it is important to remember that over time, daily academic stress may contribute to the development of long term health problems. These health problems can include hypertension and weakened immune system [1]. This may adversely affect the performance of the student. Examination stress is a psychological stress that activate hypothalamic-pituitary adrenocortical (HPA) axis to increase circulating levels of glucocorticoids [2]. Stress impairs brain functioning, which leads to learning and memory deficits. Academic examination stress among students can vary from mild to severe. These different levels of stress may affect learning performance differently. Generally, Stress is defined as nonspecific response of the body under pressure [3]. Stress can be described as acute (short term) or chronic (long term). Acute stress is the reaction to an immediate stressor. The acute physiological response to stress protects the body and brain and helps to re-established or maintains homeostasis while chronic stress is harmful. Chronic stress is defined as a state of prolonged tension from internal or external stressors, which may cause various health depressions such as Fatigue, headaches, hypertension and ulcer [3]. The measure of serum level of cortisol and progesterone is used to access the physiological stress condition of the students. Physiological response to stressors indicates that epinephrine (adrenaline), noradrenaline and cortisol, a glucocorticoid is secreted. Epinephrine which combines elements of hormones and neurotransmitters is quickly released into the bloodstream to put the body into a general state of arousal and enable it to cope with the challenge. The adrenal glands secrete glucocorticoids, which are hormones that produce an array of effects to stress. At the same time, the secretion of the reproductive hormone (progesterone) is inhibited during stress to conserve energy for the imminent fight or flight. Under normal condition, the level of cortisol is controlled by a negative feedback mechanism. However if the body is chronically exposed to stressor, the hormone in turn is constantly released. Long –term stress and elevated cortisol have been linked to insomnia, chronic fatigue syndrome, thyroid disorder, dementia, depression [4]. In

chronic stress, the body diverts pregnenolone, the precursor of progesterone, to provide for the production of cortisol a phenomenon known as pregnenolone steal whereby progesterone is shunted into the stress hormone pathway, making less progesterone available for its important role in many body functions [5].

Some pressure able factors such as Academic workload, inadequate resources, low motivation, poor performance in academic, continuous poor performance in academic, overcrowded lecture halls, and uncertainty of getting job after graduating from the University, can create academic stress among students, and they are considered stressors amongst students [6]. [7], defined academic stressors as self relevant thoughts or events that impair the accomplishment of a goal related to school performance to overall school success. Academic stressors have been linked to many assignments, competition with other students, failures, lack of financial motivation, poor relationships with other students or lecturers, family or problems at home [8]. The system of education in some developing countries where facilities do not go round for all students and where there is massive pressure on the students are further stressors that may influence performance of students. Persistent academic exercise under these conditions can induce a chronic stress which may adversely affect the performance of the students as well as their health conditions.

2. MATERIALS AND METHODS

2.1 Subjects

A total of 30 apparently healthy male and female students who were to write a day examination were selected as test subjects while 30 apparently healthy male and female students who have no examination served as control subject. The mean age of the participants was 24 years. Students with observable signs and symptoms of any sickness, those who smoke, drink alcohol or under any medication; female students in their menstrual period and pregnant women were excluded from the study. The blood samples were collected between the hours of 7am to 8am for pre-examination group and between the hours of 11am to 12noon for post-examinations group. The students that participated in the study gave informed consent before 5ml of venous blood sample was collected from the antecubital vein by 7am to 8 am before examination in the test group and control group while another 5ml of blood sample was collected from the test group by 11am to 12 noon after the examination. The examination started by 9.00am and ended by 10.00 am. Blood was allowed to clot at 4°C and serum was separated and stored at -20°C until estimation of cortisol, progesterone and albumin.

2.2 Estimation of Serum Cortisol and Progesterone

This was done using the DRG cortisol immunoassay kit (DRG International, Inc., USA). Briefly, the DRG cortisol ELISA kit is a solid phase enzyme-linked immunosorbent (assay ELISA). The microtitre wells are coated with monoclonal antibody directed towards an antigenic site on the cortisol molecule. Endogenous cortisol of a patient sample competes with a cortisol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound antibody are washed off. The absorbance is measured spectrophotometrically at 450 nm. The amount of unbound peroxidase conjugate is inversely proportional to the concentration of cortisol in the sample.

2.3 Estimation of Serum Albumin

This was done using Albumin Randox Kit (United Kingdom). The procedure was as described by the manufacturer. In brief, 10µl of sample or standard was added into 3ml of BCG reagent and allowed to stand for 10minutes and reaction read at 630nm wavelength against reagent blank.

2.4 Statistical Method

The variables were expressed as mean ± standard deviation (SD). The mean differences were assessed using paired student's t-test and independent t-test, while levels of association were assessed using Spearman's correlation coefficient. Significant level was considered at p<0.05.

3. RESULTS

The mean ± SD Pre-examination cortisol (ng/ml) value was 409.97± 146.39 while the post examination cortisol (ng/ml) value was 240.90±122 (P<.001). The mean ± SD pre-examination progesterone (ng/ml) value was 0.54 ± 0.36, while the post examination progesterone (ng/ml) value was 1.12±0.93 (P=.01) (Table 1).

Table 1. Mean (±SD) values of cortisol (ng/ml) and progesterone (ng/ml) pre and post examination

Parameter	Pre-exam	Post-exam	T	P-value
Cortisol	409.97± 147.39	240.90±122	5.249	<0.001
Progesterone	0.54±0.36	1.12±0.93	-3.565	<0.01

Key
SD=standard deviation

The mean ± SD Pre- examination cortisol (ng/ml) value was 409.97± 146.39 while the control cortisol (ng/ml) value was 203.75±135 (p<0.01). The mean ± SD pre-examination progesterone (ng/ml) value was 0.54 ± 0.36, while the control progesterone (ng/ml) value was 2.24±1.97 (p<0.01) (Table 2).

Table 2. Mean (±SD) values of pre-exam cortisol (ng/ml) and control group (ng/ml) in test subject

Parameter	Pre-exam	Control group	T	P-value
Cortisol	409.97± 147.39	203.75±135	5.500	<0.01
Progesterone	0.54±0.36	2.24±0.1.97	-4.564	<0.01

Key
SD=standard deviation

The mean ± SD Post-examination cortisol (ng/ml) value was 240.90± 122 while the control cortisol (ng/ml) value was 203.75±135 (P=.01). The mean ± SD post-examination progesterone (ng/ml) value was 1.12 ± 0.93 while the control progesterone (ng/ml) value was 2.24±1.98 (P=.01).

There was a significant negative correlation between pre-progesterone versus pre-cortisol ($r = -0.399$, $P=.05$). and a significant correlation between pre-progesterone versus post-progesterone ($r0.419$, $P=.05$). (Table 3).

Table 3. Level of associations between pre and post cortisol (ng/ml) and progesterone (ng/ml) and examination scores

Parameters (N=30)	Pre-cortisol	Post-cortisol	Pre-progest	Post-progest	P-value
Post cortisol	0.183		0.068		>0.1
Exam Scores	0.263	0.192	0.260	-1.198	>0.1
Pre-cortisol			-0.399		=.05
Post-progest.			0.419		=.05

4. DISCUSSION

Stress has become important issue in academic circle as well as in our society. The system of education in Nigeria where learning facilities do not go round for all students and where there is massive pressure on students, excess academic workload, inadequate resources, low motivation, poor performance in academic, overcrowded lecture halls, and uncertainty of getting job after graduating from the university. Sequel to the above mentioned stressors, this study reveals that serum level of cortisol increases significantly in Nigerian students who represents a good number of blacks, before examination. This may be an indication that the students were under stress before and during the examination. Apart from the clinical implications that may result from clustered examinations within few days, it is also important to note that the above mentioned stressors may persist during the whole academic period of the students. Thus chronic stress may ensue. The physiological effect of academic examination stress such as increased secretion of cortisol may be a temporary issue as the normal homeostasis returns after the stressor might have been removed. However, the surrounding pressure may make examination a dreaded circumstance.

Under many stressors, couple with the student's effort to stretch his ability and time, to meet up with his academic demands, chronic stress is set-in. More over when these academic stressors take place, an individual becomes disorganized, disoriented and therefore less able to cope with the academic demand. Cortisol is one of the major stress hormones and is released in both acute and chronic stress responses [9]. Meanwhile production and release of progesterone is inhibited to conserve energy for combating the immediate challenge [10].

This study observed that the mean value of serum cortisol is significantly higher just before the examination than after examination. This suggests that cortisol was released in response to the academic stress (examination) to restore homeostasis through regulation and mobilization of energy to meet the physiological demands placed upon the body. Likewise, the progesterone significantly dropped just before the examination thereby consolidating the fact that production and release of progesterone, a reproductive hormone, is inhibited to conserve energy. The significant increase in the serum cortisol in the test subjects before the examination compared to the control subjects supports the claim that examination is a very serious clinical stressor in Nigeria.

Under stress, hypothalamic-pituitary-adrenal (HPA) axis is stimulated which eventually culminate into production and release of corticotrophin releasing hormone [11]. The hormone is transported to its target, the pituitary gland, via the hypophyseal portal system to which it binds and causes the pituitary gland to secrete its own messenger, adrenocorticotrophic

hormone into the body's blood stream. Our finding is in accordance with those of [12] and [13] that academic stressors are effective trigger of cortisol elevation.

There was no significant difference in mean serum levels of cortisol between the post-examination and control groups. Serum cortisol levels dropped significantly after the examination. The significant difference can be attributed to both the circadian rhythm of cortisol and a negative feedback mechanism as the students feel relieved of examination anxiety after the examination. The later may be a stronger effect than the former, though we could not determine the cortisol and progesterone level of the control group during post examination time. This was partly because we could not hold back the control students at rest till the time of second sample collection. It might be necessary to carry out such study in order to test the hypothesis suggested above.

In humans, the amount of cortisol present in the blood undergoes diurnal variation; the level peaks in the early morning (approximately 8 am) and reaches its lowest level at about midnight to 4 am, or three to five hours after the onset of sleep [14]. Circadian rhythm of cortisol may go as low as 140ng/ml and as high as 700ng/ml early in the morning (approximately 8am) in the same vein, it can go as low as 80 and as high as 350 at about midnight to 4am [14]. The mean \pm SD Pre-examination cortisol (ng/ml) value of 409.97 ± 146.39 is considered to be high at diurnal sample collection while the control cortisol (ng/ml) value was 203.75 ± 135 ($P=.01$).

Though this study could not compare the cortisol levels amongst different age groups and could not differentiate sources of stressors, this study showcases the role of academic examinations among stress inducers using cortisol secretion as a stress marker. This study also practically depicted that individuals may respond differently to stress. This is based on the indication that there is no significant correlations between pre-examination serum cortisol and examination scores ($P>0.1$) and at the same time there was no correlation between post examination serum cortisol and examination scores ($P>0.1$). The progesterone level was found to be inversely proportional to serum cortisol which indicates that progesterone production is inhibited when the body is faced with stress. The possible reasons may be to restore homeostasis or may be that the pregnenolone steal phenomenon is gradually setting in or may be due to the fact that cortisol and progesterone compete for common receptors in the cells, resulting in impairment of progesterone activity. In pregnenolone steal phenomenon, progesterone available for its important role in many body functions during prolonged stress.

The effects of stress on memory include interference with one's capacity to encode and ability to retrieve information [15]. The findings in this study, is confirmed by the report of [16], which indicates that when stress occurs, the body reacts by secreting stress hormones into the blood stream. Stress can cause acute and chronic changes in certain brain areas which can cause long-term damages [16]. Over-secretion of stress hormones most frequently affects memory negatively, but in a few cases can affect it positively. In particular, the hippocampus, prefrontal cortex and the amygdala are affected [16]. One type of stress hormone responsible for affecting memory negatively is Glucocorticoids (GCs), also known as cortisol [17]. Glucocorticoids facilitate and impair the actions of stress in the brain memory process. Cortisol is a known biomarker for stress [18]. Under usual circumstances, the hippocampus regulates the production of cortisol because it has many receptors that are sensitive to these stress hormones. However, an excess of cortisol can impair the hippocampus by preventing one from being able to form a new memory and retrieving an

existing memory. These stress hormones are also hindering the hippocampus from receiving enough energy by diverting glucose levels to surrounding muscles [15].

Poor financial status, low motivation, poor and continuous poor academic performance, can fuel stress to a chronic level amongst the students. This can leave the body in a continuous state of fight-or-flight response and never reaches a state of homeostasis. Such condition could adversely affect the student's performance in school. Chronic stress does affect a person's cognitive functioning differently than a person with cognitively normal subjects versus a person with mild cognitive impairment. Chronic stress and elevated cortisol has been known to lead to dementia in elderly people [18]. The study concludes that the students showed significant evidence of pre-examination stress.

5. CONCLUSION

Stress has become important issue in academic circle as well as in our society. This study reveals that the system of education in Nigeria where learning facilities do not go round for all students and where there is massive pressure on students, excess academic workload, inadequate resources, low motivation, poor performance in academic, overcrowded lecture halls, and uncertainty of getting job after graduating from the university has constituted major stressor in academic circle. These stressors have led to significant increase in serum level of cortisol in Nigerian students who represents a good number of blacks, before examination. Apart from the clinical implications that may result from clustered examinations within few days, it is also important to note that the above mentioned stressors may persist during the whole academic period of the students. Thus chronic stress may ensue. The physiological effect of academic examination stress such as increased secretion of cortisol may be a temporary issue as the normal homeostasis returns after the stressor might have been removed. However, the surrounding pressure may make examination a dreaded circumstance.

CONSENT

All authors declare that 'written informed consent was obtained from all the student participants before this study and for publication of this case report.

ETHICAL APPROVAL

All authors hereby declare that all experiments were approved by the Faculty of Health Science and Technology Ethics Committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mathews KA Salomon K, Brady SS Allen MT. Cardiovascular reactivity to stress predicts future blood pressure in adolescence. *Psychoso med.* 2003;65:410-415

2. Qurrat-ul-Aen I, Erum S, Saida H, Darakhshan JH. Perception Of Academic Examination Stress: Effects On Serum Leptin, Cortisol, Appetite And Performance. *J Ayub Med Coll Abbottabad.* 2011;23(2)
3. Jones TL. Definition of stress eating disorders in women and children prevention stress management and treatment. Boca Raton CRS press. 2001;89-100
4. Jones DS, Quinn S. Textbook of functional Medicine. Gig Harbor Wash, institute for functional medicine. 2006;203-245
5. Vallee M, Mayo W, LeMoal M. Role of pregnenolone dehydroepiandrosterone and their sulphate esters on learning and memory in cognitive aging. *Brai Res, Brai Res Rev.* 2001;37(1-3):301-12
6. Kemeny M. *Psychoneuroimmunology.* Oxford : Oxf Uni Press Inc. 2007;92-116
7. Joseph EA, Henry O. An assessment of academic stress among undergraduate students: The case of University of Botswana. *Edu Res Rev.* 2009;4(2):063-070
8. Fairbrother K, Warn J. Work dimensions, stress and job satisfaction. *J manag Psycho.* 2003;18(1):8-12
9. Wallerius S, Rosmond T, Ljung T. Rise in Morning Saliva cortisol is associated with abdominal obesity in men: a preliminary report. *J endocrinol Invest.* 2003;26:616-619.
10. Sapolsky RM. *Why zebras don't get ulcers and updated guide to stress, stress-related diseases and coping.* New York W.H Freeman; 1998
11. Koolhaas J. Stress revisited: A critical evaluation of stress concept. *Neurosci and Biobehav Rev.* 2011;35:1291-1301
12. Richard L Falgooni P Jared G Dale E Berger SJ. Examination stress as an ecological inducer of cortisol and psychological responses to stress in undergraduate students. *Informa healthcar.* 2006;9(4):199-206
13. Lewis RL Nikolova A Chang DJ Weekes NY. Examinations and stress components of working memory. *Internat J bio Stre.* 2008;11:108-114
14. de Weerth C, Zijl RH, Buitelaar JK. "Development of cortisol circadian rhythm in infancy". *Early Hum. Dev.* 2003;73(1-2):39-52.
15. Kuhlmann, S Piel, M Wolf, OT. Impaired Memory Retrieval after Psychosocial Stress in Healthy Young Men. *J Neurosci.* 2005;25(11):2977-2982.
Stressed about exams
16. Knapman A, Heinzmann JM, Hellweg R, Holsboer F, Landgraf R, Touma C. Increased stress reactivity is associated with cognitive deficits and decreased hippocampal brain-derived neurotrophic factor in a mouse model of affective disorders. *J Psychiatr Res.* 2010;44:566-75.
17. Henckens MJ AG, Hermans EJ, Pu Z, Joels M, Fernandez G. Stressed Memories: How Acute Stress Affects Memory Formation in Humans". *J Neurosci.* 2009;29(32): 10111-10119.
18. Peavy GM, Salmon DP, Jacobson MW, Hervey A, Gamst AC, Wolfson T. Patterson TL, Goldman S, Mills PJ, Khandrika S, Galasko D. Effects of Chronic Stress on Memory Decline in Cognitively Normal and Mildly Impaired Older Adults". *Am J Psychiatr.* 2009;166(12):1384-1391.

© 2014 Chinedum et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sciencedomain.org/review-history.php?iid=278&id=19&aid=2373>