

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

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Abstract

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 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 and after examination in the test group and once in the control. 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 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 associations between pre-progesterone and pre-cortisol ($r=0.399$; $P<0.05$) and pre-progesterone versus post-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

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 weakened immune system (Mathew et al., 2003). 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 (Qurrat-ul-Aen et al., 2011). Stress impairs brain functioning, which leads to learning and memory deficits Knapman et al., 2010. 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 (Jones, 2001). 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 maintain

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 (Jones, 2001). 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), non adrenaline and cortisol a glucocorticoid are 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 (Jones and Quinn, 2006). In chronic stress, the body divert 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 (Vallee et al., 2001).

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 (Kemeny (2007, Joseph and Henry, 2009), 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 identified as to many assignments, competition with other students, failures, lack of financial motivation, poor relationships with other students or lecturers, family or problems at home (Fairbrother and Warn, 2003). The system of education in Nigeria 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.

Materials and Method

Subjects

A total of 30 apparently healthy students who were to write a day examination were selected as test subjects while 30 apparently healthy students who have no examination served as control subject. An endorsed informed consent was obtained before 5ml of venous blood sample was collected from the antecubital vein in the test group before and after the examination. While blood sample was collected from the control group once. Blood was allowed to clot at 4 °C and serum was separated and stored at -20°C until estimation of cortisol, progesterone and albumin.

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 450nm. The amount of unbound peroxidase conjugate is inversely proportional to the concentration of cortisol in the sample.

Estimation of Serum Albumin

This was done using Albumin Randox Kit USA. The procedure was as described by the manufacturer.

Statistical Method

The valuables were expressed as mean $\pm$ 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 $p < 0.05$.

Results

The mean $\pm$ SD Pre- examination cortisol (ng/ml) value was 409.97 ± 146.39 while the post examination cortisol (ng/ml) value was 240.90 ± 122 ($p < 0.001$). The mean $\pm$ SD pre-examination progesterone (ng/ml) value was 0.54 ± 0.36 , while the post examination cortisol (ng/ml) value was 1.12 ± 0.93 ($p < 0.01$) see table 1

The mean $\pm$ 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 $\pm$ SD pre-examination progesterone

(ng/ml) value was 0.54 ± 0.36 , while the control progesterone (ng/ml) value was 2.24 ± 1.97 ($p < 0.01$) see table 2

The mean $\pm$ SD Pre- examination cortisol (ng/ml) value 240.90 ± 122 while the control cortisol (ng/ml) value was 203.75 ± 135 ($p < 0.1$). The mean $\pm$ SD post-examination progesterone (ng/ml) value was 1.12 ± 0.93 , while the control progesterone (ng/ml) value was 2.24 ± 1.98 ($p < 0.01$) see table 3.

There was a significant positive correlation between pre-progesterone versus pre-cortisol ($r = 0.399$, $p < 0.05$) and a significant positive correlation between pre-progesterone versus post-progesterone ($r = 0.419$, $p > 0.05$) see table 4

Discussion

Stress has become an important issue in academic circles 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, trailing 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. 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 many days due to persistent level of serum cortisol, if the above mentioned stressors persist during academic period of the students, 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 parked circumstances hovering round the academic work may make examination a dreaded circumstance. Under many stressors, and as the student trying to stretch his ability and time, to meet up with his academic demands, thereby encouraging stress. More over when these academic stressors take place, an individual becomes disorganized, disoriented and therefore less able to cope up, thus resulting in stress related health problems. The pressure to perform well in the examination or test and time allocated makes academic environment very stressful (Divaris, 2005, Chafra, 2006). Cortisol and progesterone were determined because cortisol is one of the major stress hormones and is released in both acute and chronic stress responses (Wallerius et al., 2003). Meanwhile production and release of progesterone is inhibited to conserve energy for

combating the immediate challenge (Sapolsky, 1998). Determination of serum albumin was necessary to assess the nutritional status of the subjects. 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 acute 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 in the production and release of cortisol (Koolhass et al., 2011). The hormone is transported to its target, the pituitary gland, via the hypophyseal portal system (short blood vessels), to which it binds and causes the pituitary gland to, in turn secrete its own messenger, (adrenocorticotrophic hormone) systemically into the body's blood stream. Our findings is in accordance with those of Richard et al., (2006) and Lewis et al., (2008) that academic stressors are effective trigger of elevation of cortisol levels.

There was no significant difference in the serum levels of cortisol between the post-examination and control mean values. Serum cortisol levels dropped significantly after the examination. This no significant difference can be attributed to the circadian rhythm of cortisol and a negative feed

back mechanism since the students may have been relieved of examination anxiety. 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. All vertebrates respond to stressful situations by releasing hormones, such as adrenalin and glucocorticoids, which instantaneously increase the animal's heart rate and energy level. To understand why Sapolsky said "just look at the dichotomy between what your body does during real stress—for example, something is intent on eating you and you're running for your life—versus what your body does when you're turning on the same stress response for months on end for purely psychosocial reasons."

In the short term, Sapolsky explained, stress hormones are "brilliantly adapted" to help one survive an unexpected threat. "one mobilizes energy in his thigh muscles, increases blood pressure and turn off everything that's not essential to surviving, such as digestion, growth and reproduction. "one thinks more clearly, and certain aspects of learning and memory are

211 enhanced. All of that is spectacularly adapted if you're dealing with an acute physical stressor—a
212 real one."

213 But non-life-threatening stressors, such as constantly worrying about money or pleasing your
214 boss, as was observed amongst students, also trigger the release of adrenalin and other stress
215 hormones, which, over time, can have devastating consequences to one's health. "If you turn on
216 the stress response chronically for purely psychological reasons, you increase your risk of adult
217 onset diabetes and high blood pressure. If one is chronically shutting down the digestive system,
218 there's a bunch of gastrointestinal disorders one is more at risk for as well." Studies show that
219 long-term stress also suppresses the immune system, making you more susceptible to infectious
220 diseases, and can even shut down reproduction by causing erectile dysfunction and disrupting
221 menstrual cycles (Mark, 2007). This is in line with the significantly low level of progesterone
222 detected amongst students with increased stress hormone (cortisol).

223 Furthermore, if one is chronically stressed, all sorts of aspects of brain function are impaired,
224 including, at an extreme, making it harder for some neurons to survive neurological insults. Also,
225 neurons in the parts of the brain relating to learning, memory and judgment don't function as well
226 under stress (Mark, 2007). This could be the fate of many Nigeria students under different
227 pressureable conditions fueling academic stress.

228 The effects of stress on memory include interference with one's capacity to encode and ability to
229 retrieve information (Kuhlmann et al., 2005). The findings in this study, is confirmed by the the
230 report of Henckens et al., (2009), which indicates that when stress occurs, the body reacts by
231 secreting stress hormones into the blood stream. Stress can cause acute and chronic changes in
232 certain brain areas which can cause long-term damages (Henckens et al., 2009). 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 (Henckens et al., 2009). One type of stress hormone responsible for affecting memory negatively is Glucocorticoids (GCs), also known as cortisol (Oei et al., 2007). Glucocorticoids facilitate and impair the actions of stress in the brain memory process. Cortisol is a known biomarker for stress (Peavy et al., 2009). 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 (Kuhlmann et al., 2005).

Acute stress is a stressor that is an immediate perceived threat (Neupert et al., 2006). Unlike chronic stress, acute stress is not ongoing and the physiological arousal associated with acute stress is not nearly as demanding. There are mixed findings on the effects of acute stress on memory. One view is that acute stress can impair memory, while others believe that acute stress can actually enhance memory. For acute stress to enhance memory certain circumstances must be met. First, the context in which the stress is being perceived must match the context of the information or material being encoded (Smeets et al., 2007). Second, the brain regions involved in the retrieval of the memory must match the regions targeted by GCs (Smeets et al., 2007). There are also differences in the type of information being remembered or being forgotten while being exposed to acute stress. In some cases neutral stimuli tend to be remembered, while emotionally salient stimuli tend to be forgotten. In other cases the opposite effect is evident (Jelicic et al., 2004). What seems to be an important factor in determining what will be impaired

and what will be enhanced is the timing of the perceived stressful exposure and the timing of the retrieval. For emotionally salient information to be remembered, the perceived stress must be induced before encoding, and retrieval must follow shortly afterwards (Smeets et al., 2007). In contrast, for emotionally salient stimuli to be forgotten, the stressful exposure must be after encoding and retrieval must follow after a longer delay (Smeets et al., 2007). Acute stress does affect memory formation in humans in an unexpected way. If stressful information is relatable to a person, they are more prone to store the event in permanent memory. When a person is under stress, the sympathetic system will shift completely towards a tonically active state. Acute stress can also affect a person's neural correlates which interfere with the memory formation. During a stressful time, a person's attention and emotional state may be affected which could hinder the ability to focus while processing an image. Stress can also enhance the neural state of memory formation (Smeets et al., 2007).

Chronic stress is a stressor that is ongoing for a long period of time. When chronic stress is experienced, our body is in a state of continuous physiological arousal (Pasquali, 2006). Normally, our body activates our fight-or-flight-response, and when the perceived stress is over our body returns to a state of homeostasis. When chronic stress is perceived, however, the body is in a continuous state of fight-or-flight response and never reaches a state of homeostasis. The physiological effects of chronic stress can negatively affect memory and learning. One study used rats to show the effects of chronic psychosocial stress on memory by exposing them to a cat for five weeks and being randomly assigned to a different group each day (Parkad et al., 2001). Their stress was measured in a naturalistic setting by observing their open field behaviour as well as memory for the radial arm water maze (RAWM). In the RAWM, rats are taught the place of a platform that is placed below the surface of the water. They must recall this later to discover the

platform to exit the water. It was found that the rats exposed to chronic psychosocial stress could not learn to adapt to new situations and environments, and had impaired memory on the RAWM (Parkad et al., 2001).

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 (Peavy et al., 2009).

298 **Table 1**

299 Mean ($\pm$ SD) values of cortisol (ng/ml) and progesterone (ng/ml) pre and post examination

300	Parameter	Pre-exam	Post-Exam	T	P-value
301	Cortisol	409.97 $\pm$ 147.39	240.90 $\pm$ 122	5.249	<0.001
302	Progesterone	0.54 $\pm$ 0.36	1.12 $\pm$ 0.93	-3.565	<0.01

303 Key

304 SD=standard deviation

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Table 2

Mean ($\pm$ SD) values of pre-exam cortisol (ng/ml) and pre-exam progesterone (ng/ml) in test Subject compared with control subjects

Parameter	Pre-exam	Post-Exam	T	P-value
Cortisol	409.97 $\pm$ 147.39	203.75 $\pm$ 135	5.500	<0.01
Progesterone	0.54 $\pm$ 0.36	2.24 $\pm$ 0.1.97	-4.564	<0.01

Key

SD=standard deviation

Table 3

Mean ($\pm$ SD) values of post-exam cortisol (ng/ml) and post-exam progesterone (ng/ml) and Albumin (g/l) in test Subject compared with control subjects

Parameter	Pre-exam	Post-Exam	T	P-value
Cortisol	240.90 $\pm$ 122	203.75 $\pm$ 135	1.089	>0.1
Progesterone	1.12 $\pm$ 0.93	2.24 $\pm$ 0.1.97	-2.797	<0.01
Albumin	44.73 $\pm$ 5.33	45.92 $\pm$ 6.60	-0.753	>0.1

Key

SD=standard deviation

Table 4

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

Parameters	N	Correlation	P-value
Pre-cortisol vs Post cortisol	30	0.183	>0.1
Pre-cortisol vs Scores	30	0.263	>0.1
Post-cortisol vs Exam scores	30	0.192	>0.1
Pre-progest vs Pre-cortisol	30	0.399	>0.05
Pre-progest vs Exam scores	30	0.260	>0.1
Post-progest vs Exam scores	30	-1.198	>0.1
Pre-progest vs Post-progest.	30	0.419	>0.05
Pre-progest vs Post-cortisol	30	0.068	>0.1

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UNDER PEER REVIEW