International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Pre- and Post-Examinational Stress Effect on Anti-Oxidant Level and Plasma Glucose Level of Students of College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria

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ABSTRACT

This study investigated the effect of pre- and –post examinational stress on antioxidant and plasma glucose levels in apparently healthy students. A total of 50 subjects aged 18-30 years, were recruited for the study. A structured questionnaire was used to obtain the demographic data and dietary pattern of subjects whereas 5mls of blood sample was collected from the subjects and used for the analysis of biochemical parameters. Malondialdehyde (MDA) was determined by thiobarbituric acid method, glutathione peroxidase (GPx) by Rotruck's method, Vitamin E by Desai's method and glucose by glucose oxidase method. There was no significant difference in the mean serum levels of MDA, Vitamin E, plasma glucose and GPx activity (P>0.01) before and after examination stress. The results showed no statistically significant difference when pre examination plasma glucose level was correlated with pre examination antioxidant status (P>0.01). Again, there was no significant difference on the post examination plasma glucose level when correlated with post examination antioxidant status (p>0.01). However, the result did show a significant difference when post examination MDA was correlated with post examination GPx (r=0.370; p=0.01) but there was no significant difference on the post examination vitamin E level. Therefore, examination stress when properly managed may pose no deleterious effect on the students and hence, students should seek to acquire stress management skills that will enable them cope with the challenging nature of their academic life.

Key Words: Examination, Stress, Glutathione peroxidase, malondialdehyde, Vitamin E, Glucose.

INTRODUCTION

Academic stress can be conceptualized as a student's interactions between environmental stressors, the student's cognitive appraisal of and coping with the academic-related stressors, and psychological or physiological response to the stressors (Lee and Larson, 2000; Lou and Chi, 2000). It is a pervasive problem across countries, cultures, and ethnic

groups, and must be viewed in its context (Wong *et al*., 2006). Every student aspires to pursue academic success to achieve respect, family pride, and social mobility (Gow *et al.,* 1996; Alam, 2001). This results in extremely high academic demands and extraordinary pressure on students and especially adolescents. As a consequence of stress and demands to perform well in examination the students are not able to enjoy their academic life and it becomes joyless and burden for them. Examination stress refers to the pressure or stresses that are experienced by students to perform well in final school or undergraduate examinations and competitive college entrance examinations (Fisher, 1994; Manjula and Vijaylaxmi, 2012). Having examination stress is a common and widespread phenomenon for students (Gow *et al.,* 1996; Banerejee, 2001). Up to 30%-50% of students have test-induced anxiety problems (Kahlon, 1993). A medical student in his or her life goes through several academic stresses. Singh et al. (2012) in their study have defined stress as "a physical or psychological stimulus that can produce mental or physiological reactions that may lead to illness." (Singh *et al.*, 2012). Mild stress may be beneficial in cognitive tasks and performance while persistently high stress may lead to anxiety and depression, which are definable neuropsychiatric disease entities (Vander, 2001; Shibata and Kobayashi, 2008).

But if the students feel intense stress before and during examination, it has consequences for mental health and somatic symptoms (Lee and Larson, 2000). As per the report of Banerjee's (2001), every year about 25,000 students in the age group of 18 to 20 years commit suicide during the examination month (i.e. March to June). Due to high examination stress, students spent less time in socializing and get engaged in passive and active leisure which may further magnify the effects of examination stress (Lee and Larson, 2000). Long-term exposure to stress can lead to serious health problems, chronic stress can disrupt vital systems in the body with serious effects ranging from hypertension, heart attack and stroke to suppressing immune system, enhancement of aging process due to consequent occurrence of oxidative stress and degenerative diseases (Edmunds, 1984; Banerjee, 2001).

In the last couple of decades, there has been an increase in the global prevalence of degenerative or chronic diseases such as diabetes mellitus, hypertension, cancer, Alzheimer's disease, atherosclerosis and heart disease (Doll, 1995; Albright,2008). These diseases are now the major causes of death globally (Doll, 1995; Albright, 2008). Recent evidence implicates the role of oxidative stress in the pathogenesis and/or complications of these disorders (Shibata and Kobayashi, 2008; Kadenbach *et al.*,2009). Oxidative stress is defined as an "imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage" (Sies, 1991). It is caused by increased production and/or reduced removal of reactive species by the antioxidant defenses. Oxidative stress causes oxidative damage - "the biomolecular damage caused by attack of reactive species upon the constituents of living organisms". Oxidative damage to cellular components impairs physiological functions. Reactive species may be produced in response to inflammation during which phagocytes release ROS to kill invading bacteria (Sies, 1991; Halliwell, 2011). The ability of cells to scavenge excess reactive species is largely dependent on the efficiency of the overall antioxidant defense system (Halliwell, 2011).

An antioxidant has been defined by Halli well as "any substance that, when present at low concentrations compared with those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate". A simplified definition is "any substance that delays, prevents or removes oxidative damage to a target molecule" (Moore *et al.*,1992). The antioxidant defense includes both

antioxidants produced in the body (endogenous) and antioxidants derived from the diet (exogenous). The endogenous antioxidants are found both intracellularly and extracellularly. Uric acid, ubiquinol, glutathione and the metal binding proteins albumin, transferrin and ferritin are examples of non-enzymatic endogenous antioxidants. Superoxide dismutase (SOD), catalase and glutathione peroxidase(GSH-Px) are examples of enzymatic endogenous antioxidants. Exogenous antioxidants are provided by the diet and include vitamins and other antioxidative plant compounds but also essential minerals required in the active site of antioxidative enzymes such as selenium in GSH-Px. The antioxidant defense is considered to be a complex integrated system where substances are suggested to interact synergistically. Academic stress is a pervasive problem across countries, cultures, and ethnic groups, and must be viewed in its context (Wong *et al.,* 2006). Up to 30%-50% of students have test-induced anxiety problems (Kahlon, 1993). Every year about 25,000 students in the age group of 18 to 20 years commit suicide during the examination month (i.e. March to June) (Banerjee, 2001). Due to high examination stress, students spent less time in socializing and get engaged in passive and active leisure which may further magnify the effects of examination stress (Lee and Larson, 2000). Long-term exposure to stress can lead to serious health problems, chronic stress can disrupt vital systems in the body with serious effects ranging from hypertension, heart attack and stroke to suppressing immune system, enhancement of aging process due to consequent occurrence of oxidative stress and degenerative diseases. The declaration of Helsinki states that, "the purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease" (Declaration of Helsinki, 1964).Although studies have been done on pre- and post-examinational stress effect on anti-oxidant level and plasma glucose level, this study will further advance the knowledge of this relationship, thereby helping to provide vital information needed by clinicians in the diagnosis and management of patients in our locality.

Inclusion and Exclusion Criteria

Male students who are non-alcoholic, non-smokers and non-diabetic between 18 and 30 years not on active exercise were recruited for this study while Male students above the age of 30 years with diabetes, smoking habits and alcoholism and actively exercising were excluded from the study.

MATERIALS AND METHODS

Study Design

The research was designed to evaluate the pre- and –post examination stress effect on antioxidants and plasma glucose level in apparently healthy students of Nnamdi Azikiwe University, Nnewi Campus, Anambra State. A total of 50 subjects were randomly recruited for this study. Five milliliters (5ml) of whole blood was collected by standard venepuncture. Two milliliters (2mls) was dispensed into fluoride oxalate container and used for the blood glucose estimation while the remaining was dispensed into plain tube, allowed to clot and centrifuged and serum separated for anti-oxidant estimation. Plasma glucose, malondialdehyde**,** glutathione peroxidase and vitamin E levels were estimated.

Laboratory Methods

MDA estimation was determined using thiobarbituric acid method as described by Ohkawa *et al*., 1979; Gpx was determined by Rotruck's method as described by Hafemann *et al.*, 1974; Vitamin E was determined using Desai's method as described by Baker and Frank, 1968 while plasma glucose was estimated using oxidase method as described by Bergmeyer and Brent, 1974.

Ethical Consideration

The ethical approval for this research was obtained from Ethics Committee of Faculty of Healthy Sciences and Technology, NnamdiAzikiwe University in accordance with the Helsinki declaration by the World Medical Association (WMA) on the ethical principles for medical research involving human subjects (*Levine and Robert, 2006*). Informed consent was obtained from the subjects before sample collection.

Statistical Analysis

Statistical package for social science (SPSS) version 20 was employed in the analysis of the result and the data obtained for different parameters expressed as mean± standard deviation. Parameters were compared between different groups using Pearson correlation. Level of significance set at P=0.01.

RESULTS

The mean ± SD Pre-examination plasma glucose level (mmol/l) was 5.36±2.02 while the post examination plasma glucose level (mmol/l) was 4.05+1.57, this was not significant at (P=0.01). The mean ± SD Pre-examination malondialdehyde (MDA) (nmol/ml) level was 2.02±0.59 while the post examination malondialdehyde (MDA) (nmol/ml) level was 1.38±0.58, which was not significant at (P=0.01). The mean ± SD Pre-Examination Glutathione (GSH) (µ/l) level was 0.59±0.19while the post examination Glutathione (GSH) (µ/l) level was 0.93±0.25, which was not significant at (P=0.01). The mean ± SD Pre-Examination Vitamin E (µmol/l) level was 4.78±1.30 while the post examination Vitamin E (µmol/l) level was 6.01±1.24, which was not significant at (P=0.01). (See table 1 below)

Table 1: Pearson Correlation between the pre and post values of the antioxidant status and plasma glucose level among the subjects.

Parameters	Pre mean±SD	Post mean±SD	r-value	P -value
Glucose	5.36±2.02	4.05±1.57	0.156	0.291
MDA	2.02±0.59	1.38±0.58	-0.035	0.814
GSH	0.59±0.19	0.93±0.25	0.097	0.514
VITE	4.78±1.30	6.01±1.24	0.178	0.226

***Statistically significant at P=0.01**

Table 2 shows a non-statistically significant difference when pre examination plasma glucose level was correlated with pre examination antioxidant status, with r-value of 0.155, 0.166 and -0.160 for malondialdehyde (MDA), Glutathione (GSH) and Vitamin E respectively. There was no significantly difference on the post examination plasma glucose level when correlated with post examination antioxidant status, with r-value of 0.246, 0.274 and 0.241 for malondialdehyde (MDA), Glutathione (GSH) and Vitamin E respectively.

Table 2: Pearson correlation between pre and post plasma glucose level and pre and post antioxidant status among subjects.

Parameters		r-value	p-value
Pre plasma Glucose	MDA	0.155	0.293
	GSH	0.166	0.260
	VITE	-0.160	0.277
Post plasma Glucose	MDA	0.246	0.092
	GSH	0.274	0.060
	VITE	0.241	0.099

***Statistically significant at P=0.01**

Table 3 shows a significantly difference (p=0.01) when post examination malondialdehyde (MDA) was correlated with post examination Glutathione (GSH) with an r-value of 0.370, but there was no significantly difference on the post examination vitamin E level. The pre examination malondialdehyde (MDA) when correlated with pre examination Glutathione and Vitamin E, showed no significant difference.

Table 3: Pearson correlation between post and pre MDA with glutathione peroxidase activities and vitamine E among subjects.

Parameters		r-value	p-value
Post MDA	GSH	0.370**	0.010
	VITE	0.124	0.401
Pre MDA	GSHS	0.94	0.542
	VITE	-0.65	0.659

** correlation is significant at 0.01 (2 tailed)

DISCUSSION

Academic examinations have been reported to have a significant impact on the student's well-being (Loft *et al.*, 2007).

In the present study, the result showed that there was no statistical difference on the mean plasma post

examination glucose level (4.05±1.57)of the students when compared with their baseline(5.36±2.02) (P>0.01); see table 1. This is in line with the report of Maduka *et al.* (2015) who investigated the relationship between serum cortisol, adrenaline, blood glucose and lipid profile of undergraduate students under examination stress and found that there was no significant difference in the mean serum level of glucose before and during examination (P>0.05). This may be as a result of the short term duration of students' exposure to academic/examination stress.

Interestingly, Examination stress had no significant effects in the mean serum levels of malondialdehyde (MDA), Vitamin E as well as Glutathione peroxidase (GPx) level in subjects before and after examination stress (P>0.01). This is in agreement with the study carried out by Kyamon *et al.* (2017) in which they investigated the Cortisol, β-endorphin and oxidative stress markers in healthy medical students in response to examination stress and found that the malondialdehyde (MDA) and Glutathione peroxidase (GPx) levels were not statistically significant in subjects studied after examination (P>0.05). Again, the result shows a non-statistically significant difference when pre examination plasma glucose level was correlated with pre examination antioxidant status, with r-value of 0.155, 0.166 and -0.160 for malondialdehyde (MDA), Glutathione peroxidase (GPx) and Vitamin E respectively (P>0.01). There was no significant difference on the post examination plasma glucose level when correlated with post examination antioxidant status, with r-value of 0.246, 0.274 and 0.241 for malondialdehyde (MDA), Glutathione peroxidase (GPx) and Vitamin E respectively (P>0.01).

However, there was a significant difference (p=0.01) when post examination malondialdehyde (MDA) was correlated with post examination Glutathione peroxidase (GPx) with r-value of 0.370, but there was no significantly difference on the post examination vitamin E level. The pre examination malondialdehyde (MDA) when correlated with pre examination Glutathione peroxidase and Vitamin E, showed no significant difference.

In conclusion, the results revealed that there were no significant differences in the biochemical parameters studied and this may be dependent on the duration of examination stress. Based on the findings, examination stress when properly managed may pose no deleterious effect on the students' mental and social well-being and hence, students should seek to acquire stress management skills that will enable them cope with the challenging nature of their academic life and environment.

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How to cite this article: Ihim AC, Abiodu BE, Ukibe NR et al. Pre- and post-examinational stress effect on anti-oxidant level and plasma glucose level of students of college of health sciences, Nnamdi Azikiwe university, Nnewi campus, Anambra state, Nigeria. Int J Health Sci Res. 2017; 7(11):32-37.

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