EFFECT OF EXAMINATION STRESS ON OXIDATIVE STRESS MARKERS AND CORTISOL LEVELS OF MEDICAL STUDENTS

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ABSTRACT

Examination stress is a basic factor that may affects many organ and systems of the body. There is a relation between stress and biochemical and physiological markers. This study examined the level of oxidative stress markers and cortisol levels in medical students during and after examinations. Cross-sectional study was conducted at the College of Medicine Chukwuemeka Odumegwu University, Uli. Forty (40) healthy medical students planning to take their professional exam were recruited. Students with health problems that may alter immune oxidative stress measures were excluded from the study. Three samples were taken each from the students at 6-months and 2 days prior to the examination, and third sample collected two week after the examination. Eight milliliters (8 ml) of blood samples were collected from all subjects by sterile disposable. The analysis of oxidative stress markers (superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA) and total antioxidant status (TAS)) were determined by standard colorimetric method, while analysis of cortisol was done using enzyme-linked immunosorbent assay (ELISA) technique. The mean levels of MDA and cortisol were significantly higher in 2 days pre exam condition than 6 months pre and one week post exam conditions, also the activities of SOD, GPx and TAS were significantly lower in 2 days pre examination conditions, but the difference between the 6 months pre examination state and two weeks post exams state was non-significant except in the cortisol level. Examination in medical school are stressful enough to produce changes in the levels of antioxidants and cortisol level. Though the changes has a transient effect on deranging the biochemical marker.

Key words. Examination stress, Cortisol, Oxidative stress markers.

INTRODUCTION

Examination stress is a common condition faced by all students prior to and during examinations and is quite prominent among the medical students. Medical education course curriculum which entails tedious classroom work, practical sessions and clinical rounds coupled with the pattern of the examinations, fear of failure, inability to cope with first exposure to a very different system of education and competition among classmates are extremely stressful condition for students (Benoit *et al.,* 2001; Shah and Trivedi, 2009). The curriculum used by the medical students is vast and the time in which they have to complete their studies before their 2nd MBBS examination is short, usually 18 months of three semesters in most Nigerian Universities. Therefore, medical students are forced to be under stress, especially before their examinations.

Stress is defined as a mental or emotional strain resulting from very demanding circumstance, it is characterized by depression, anxiety, hysteria and worries (MacEwen, 2008). Students have shown that they experience academic stress at predictable times each semester with the greatest sources of academic stress resulting from taking and studying especially medical examinations, grade competition, and the large amount of content to master in a small amount of time (Archer and Lamnin, 1985; Abouserie, 1994; Britton and Tesser, 1991).

Stress causes an imbalance of the parasympathetic and sympathetic nervous system due to psychic stimuli which lead to disturbance of homeostasis in the body (Dvivedi, 2010). Stress of any form is known to produce definable mental and physiological reactions in the body like alterations in different biological functions especially the heart rate and blood pressure.

Stress may cause alterations in homeostasis, while inducing series of biochemical changes (Ifemeje *et al*., 2015; Egbuna and Ifemeje, 2017; Egbuna, 2018). This alterations in severe situations, may lead to challenging situation, and the body may respond by releasing some hormones such as cortisol and adrenaline. This may increase peripheral resistant and increase the blood pressure. Furthermore, reactive oxygen species may be released in severe conditions. The imbalance between generation of reactive oxygen species and its neutralization lead to oxidative stress (Egbuna and Ifemeje, 2017).

In recent years, traumatic episode during examinations and subsequently poor results following the examination have made stress an issue of major concern in academics. However, fewer studies have been conducted on health challenges of medical students undergoing examinations due to stress.
Therefore, this research attempted to evaluate the effect of examination related stress on oxidative stress markers and cortisol during University examination among young healthy MBBS students of Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State.

MATERIALS AND METHOD

The present study was carried out at College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria. Third year MBBS students of 2016-2017 batch were selected for the study. Out of 45 male medical students, 40 male students who consented after using exclusion criteria were recruited for the study. The male students were chosen because of the influence of menstrual cycle of female students on cortisol. Six months prior to the 2nd MBBS examination, (the students being unaware of the forthcoming examination), baseline blood samples were taken for MDA, SOD, GPx, TAS and cortisol. Then 2 days prior to 2nd MBBS examination, the students were invited to the department. After explaining the purpose of the study the second blood sample was taken for analysis. Two weeks after the 2nd MBBS examination the final blood sample was collected for analysis of the measured parameters.

Calculation of Sample Size

Sample size calculation was done using 95% confidence interval with 0.05 precision. Academic stress and health has been reported to occur in 2.7% (Smith *et al.*, 2000).

$$n = \frac{Z^2 pq}{d^2}$$

Where, $q = 1 - p$

n = sample size, Z=standard normal deviation at 95% confidence interval which is 1.96

p= proportion of the target population (estimated at 2.7% which is 2.7/100 = 0.027)

Q= alternate proportion (1-p) which is 1-0.027= 0.973, d= desired level of precision or significance (0.05)

Substituting the values,

$$n = \frac{1.96^2 \; x \; 0.027 \; (1 - 0.027)}{0.5^2}$$

$$= \frac{3.84 \; x \; (0.027)(0.973)}{0.0025}$$

$$= 39$$

Sampling Method

The sampling technique employed was convenient sampling method and it involved the use of questionnaire which was personally administered.

Samples Collection and Assay

Eight milliliters (8 ml) of blood samples were collected from all subjects by sterile disposable syringes into a sterile plain container and allowed to clot, retracted and centrifuge at 3000 rpm for 10 minutes. Thereafter, the serum was separated into two aliquots. One part of samples were stored at -20 ˚C until analysis of oxidative stress markers (SOD, GPx and MDA) within two weeks of collection, while the remaining aliquot was used for analysis of cortisol. The analysis were done at Chemical Pathology Department, Nnamdi Azikiwe University teaching Hospital Nnewi.

Statistical Analysis

The collected data and the laboratory results were computed. Statistical analysis was done using SPSS version 21. The quantitative results were expressed as means ± standard deviation (SD). ANOVA was used to determine the difference between the 6 months pre-examination, 2 days pre examinations and two week post- examination conditions. P value < 0.05 will be considered significant.

Methods of analysis

Cortisol estimation using Enzyme Linked Immunosorbent Assay by Foster and Dunn (1974).
TAS estimations using Colorimetric method by Koracevic *et al.* (2001).
SOD was assayed by colorimetric method of Misra and Fredovich (1972).
The activity of glutathione peroxidase was determined the method of Rotruck *et al.* (1973).
MDA level was determined by the colorimetric method of Gutteridge and Wilkins (1982).

RESULTS AND DISCUSSION

Our study was restricted among 3rd year professional MBBS students of Chukwuemeka Odumegwu Ojukwu University, Uli. This restriction is because the 2nd MBBS professional examination is assumed to be more stressful than other professional examinations.

Stress before and during examination encountered by medical students is a well-known phenomenon in all the medical school worldwide. This may be worst in developing countries where poor facilities and environmental factors have a higher attributes. Physiological studies have shown that stress from any source can influence on the endocrine, hemopoietic and immune system. A stress induced increase in cortisol levels has been reported to accelerate glucose metabolism and the production of ROS (Şimşek *et al.*, 2016).

From our study, the serum cortisol levels of 2 day pre exam condition were significantly higher when compared with the 6 months pre and 2 weeks post examination conditions (Table 1). Physical and psychological stressors cause release of glucocorticoids; these substances play an important adaptive role, mobilizing fuel inform of ATP to tissues that require energy and suppressing unessential anabolism. However, when 6 month pre exam condition were compared with the post examination condition, the mean level was significant. This could be as a result of either the fear of failing the examination or the initial rise of the cortisol during the examination which has not fallen to its normal level. Some authors have suggested that the stress of examinations induce elevated activity in the hypothalamic–pituitary–adrenal (HPA) axis and increased release of cortisol (Frankenhaeuser *et al*., 1978; Lovallo *et al.,* 1986; Malarkey *et al.,* 1995; Lucini *et al.*, 2002). However, some authors discovered either no change in cortisol secretion or even decreased release of cortisol in the course of examinations (Glaser *et al,*. 1994; Vedhara *et al*., 2000). One explanation for these variations in findings may be that the extent to which a stressor induces an elevation in cortisol is dependent on a variety of factors which include novelty, uncertainty and negative emotions (Mason, 1968; Dickerson and Kemeny, 2004).

Table 1: Mean levels of Oxidative Stress Markers and Cortisols of Students Pre and Post Examination Conditions

Exams Conditions	Cortisol (ng/ml)	TAS (mmol/l)	MDA (nmol/L)	GPx (µmol/min)	SOD (µmol/min)
Pre Exams 6 months (A)	22.60±8.07	1.80±0.41	1.67 ± 0.85	1.10 ± 0.09	15.85±1.55
Pre Exams 2 days (B)	34.84±8.57	0.67±0.08	2.10±0.65	0.95±0.085	13.45 ± 1.64
Post Exams 2 weeks (C)	24.55±10.21	1.60±0.06	1.96±0.58	1.00±0.080	14.10±1.93
P Value	<0.005*	<0.05*	<0.05*	<0.05*	<0.05*
A/B	0.015	0.001	0.001	0.031	0.03
A/C	0.021	0.31	0.25	0.83	0.63
B/C	0.001	0.029	0.042	0.58	0.55

*The mean difference is significant at the 0.05 level.

In our study, the levels of MDA and TAS were significantly higher during the 2 day pre exams period when compared with the 6 months pre exam and 2 weeks post exam condition This is also supported by the pronounced depression in SOD and GPx activities which suggest that severe stress conditions may lead into a mechanism involving oxidative stress. SOD and GPx are among machinary of biological antioxidant systems that protect membranes from oxidative damage by reactive oxygen species. It has been shown that emotional stress may induce oxidative damage, and considerably change the balance between pro-oxidant and antioxidant factors in the brain. Exposure to stress situations has been proposed to impair antioxidant defenses, leading to oxidative damage by changing the balance between oxidant and antioxidant factors (Qamber *et al*., 2018). Their findings were in consistence with our study.

CONCLUSION

Examinations in medical school are stressful enough to alter some biochemical processes in the system. Examination is a stressor and it is associated with decrease in total antioxidants status, anti-oxidant enzymes that could lead to depletion in the total antioxidant status and possibly lead to free radical mediated cell

damage in multiple days examinations. These alterations when severe may lead to deleterious effect in some vital organs.

REFERENCES

Abouserie, R. (1994). Sources and level of stress in relation to locus of control and self-esteem in university students. *Educational Psychology,* 14**:** 323-330

Archer, J. and Lamnin, A. (1985). An investigation of personal and academic stressors in college campuses. *Journal of College Student Personnel*, 26: 210-215.

Benoit D., Esa L. and Ralph G. (2001). The Driving Test as a stress Model: Effects on the blood picture, serum cortisol and the production of interleukins in man. *Life Sci*; 68 (14):1641-7.

Britton, B.K. and Tesser, A. (1991). Effects of time-management practice on college grades. *Journal of Educational Psychology,* 83: 405-410.

Dvivedi J. (2010). Managing lifestyle disorders: the physiological remedy. Proceedings of 56th National Annual Conference of APPICON.

Egbuna, C. (2018). Antioxidants and Phytochemicals. In: *Phytochemistry, Volume 2: Pharmacognosy, Nanomedicine, and Contemporary Issues*. Egbuna, C. et al. (Eds). Apple Academic Press: New York. Pp. 131-146.

Egbuna, C. and Ifemeje, J.C. (2017). Oxidative Stress and Nutrition. *Tropical Journal of Applied Natural Sciences*, 2(1): 110-116. Doi: https://doi.org/10.25240/TJANS.2017.2.1.19.

Foster, L. and Dunn, R. (1974). Single antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. *Clinical Chemistry,* **20**:365-368.

Frankenhaeuser, M., von Wright, M., Collins, A., vonWright, J. and Sedvall, S.C. (1978). Sex differences in psychoneuroendocrine reactions to examination stress. *Psychosom Med* 40:334–343.

Glaser, R., Pearl, D.K., Kiecolt-Glaser, J.K. and Malarkey, W.B. (1994). Plasma cortisol levels and reactivation of latent Epstein–Barr virus in response to examination stress. *Psychoneuro endocrinology* 19:765–772.

Gutteridge, J.M.C. and Wilkins, S. (1982). Copper-dependent hydroxyl radical damage to ascorbic acid formation of a thiobarbituric acid- reactive product. 137 (2): 327–330

Ifemeje, J.C., Udedi, S.C., Okechukwu, A.U., Nwaka, A.C., Lukong, C.B., Anene, I.N., Egbuna, C. and Ezeude, I.C. (2015). Determination of Total Protein, Superoxide Dismutase, Catalase Activity and Lipid Peroxidation in Soil Macro-fauna (Earthworm) from Onitsha Municipal Open Waste Dump. *Journal of Scientific Research & Reports*, 6(5): 394-403. DOI: 10.9734/JSRR/2015/12552.

Kemeny, M.E. and Laundenslager, M.L. (1999). Beyond stress: The role of individual difference factors in psychoneuroimmunology. *Brain Behav Immun* 13:73– 75.

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S. and Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. *Journal of Clinical Pathology,* 54: 356-361.

Lovallo, W.R., Pincomb, G.A., Edwards, G.L., Brackett, D.J. and Wilson, M.F. (1986). Work pressure and the type A behavior pattern in exam stress in male medical students. *Psychosom Med* 48:125–133.

Lucini, D., Norbiato, G., Clerici, M. and Pagani, M. (2002). Hemodynamic and autonomic adjustments to real life stress conditions in humans. *Hypertension* 39:184–188.

Malarkey, W.B., Pearl, D.K., Demers, L.M., Kiecolt-Glaser, J.K. and Glaser, R. (1995). Influence of academic stress and season on 24-hour mean cortisol concentration of ACTH, cortisol, and b-endorphin. *Psychoneuro endocrinology* 20:499– 508.

Mason, J.W. (1968). A review of psychoendocrine research on the pituitary–adrenalcorticalsystem. *Psychosom Med:* 30: 576– 607.

Misra, H.P. and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biol Chem.*; 247(10):3170–3175.

Qamber, J.H., Shah B.G., Sajjad. S. and Ibrahim, K.M. (2018). Assessment of Oxidative Stress Markers in medical students in response to examination stress. *Pakistan Journal of Medical and Health Sciences* 12(2):804-806.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W.G. (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science* 179:588-90.

Sanjay, K., Kunal, D.K. and Jha, S.D. (2016). Effect of stress during university examination on the differential leucocyte count (DLC), Heart Rate (HR), and Blood Pressure (BP) .*Indian Journal of Clinical Anatomy and Physiology*, 3(2); 163-166

Shah, C. and Trivedi, R.S. (2009). Coping of stress by medical students. *Journal of Clin Diag Res;* 19:401-523.

Şimşek, Ş., Yüksel, T., Kaplan, İ., Uysal, C. and. Aktaş, H. (2018). The levels of cortisol and oxidative stress and DNA damage in child and adolescent victims of sexual abuse with or without posttraumatic stress disorder," Psychiatry Investigation, vol. 13, no. 6, pp. 616–621.

Smith, A., Caroline, B., Alison, C., Victoria, M., Rachel, M. (2000). The Scale of Occupational Stress: A further analysis of the impact of demographic factors and type of job. Health and Safety Executive. Pg: 1-72.

Vedhara, K., Hyde, J., Gilchrist, I.D, Tytherleigh, M. and Plummer, S. (2000). Acute stress, memory, attention and cortisol. *Psychoneuro-endocrinology* 25:535–549.