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Age-Related Changes In Stress And Neuromuscular Markers Before And After Sleep Period Among Male Subjects In Elele, Rivers State, Nigeria.

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ABSTRACT
Stress is a physiologic response that involves the nervous, endocrine and immune system. Age-related biochemical changes in response to stress is not clearly understood. This study investigated the changes caused by stress signals on some biochemical markers of physical activity and higher intellectual functions in different ages. The samples were male subjects sampled randomly. Statistical significance was tested at 95% confidence interval. Biochemical markers were tested in blood samples, 1 hour before onset and after sleep period. There was a significant increase in stress hormones like cortisol, growth hormone and thyroxine in all age groups from 20-24 years, with a significant increase observed in age group 22-24 compared to 12-14. There was an increase in cortisol in same groups in the interval after sleep. Growth hormone was higher before compared to after sleep. Thyroxine level was higher after sleep in ages 18 to 22 years but higher before sleep in ages 22 to 24 years. There was a significant increase in creatine kinase, acetylcholinesterase and glutamate in age groups 18-24 years with a significant increase in age group 22-24 compared to 12-14. Creatine kinase was higher before sleep compared to after sleep but the reverse was observed in acetylcholinesterase and glutamate. The study showed that stress differs with age, an hour before and after sleep period.

Key words:*Age; Stress; Sleep; Nervous; Hormones*

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I. INTRODUCTION

The body responds to stress through rapid, intermediate and delayed mechanisms [1-2]. These mechanisms are intended to enable resistance [3] and reversal of the body to normal [3-4]. All vital physiologic systems of the human body are inherently programmed, through rigorous fine-tuning processes achieved during evolution with the aim of preserving a predefined and almost steady state [5-7]. The almost steady state of an organism is essential for its well-being and survival [8]. This optimal steady state is frequently challenged by intrinsic and extrinsic adverse forces, real or even perceived, and are described as stressors [9]. The body's response during stress is mediated by an interconnected and complex neuroendocrine, cellular and biomolecular architecture, located in both the central (CNS) and peripheral nervous systems (PNS) [10]. The adaptive response of every individual to particular stressors can be determined by a multiplicity of genetic, developmental and environmental factors [10-11]. Humans may sleep to relieve stress or stress-induced changes which can be physical or psychological [12]. Prolonged sleep deprivation has been proven to precipitate the manifestations of chronic stress exposure in a controlled study [13-14]. The inability to respond effectively to stress may lead to various diseases [13-15]. This study aimed at investigating the age-related changes age-related changes in stress and neuromuscular markers before and after sleep period

II. MATERIALS AND METHODS

Ethical approval

This study was approved by Madonna University Research Ethics Committee. All test subjects were sampled in strict confidentiality and adherence to the use of human subjects in scientific research.

Population of study

The study population was within the matrices of Elele, in Ikwerre Local Government Area of Rivers State, Nigeria. Only individuals with at least five years' residency were included as samples.

Sample collection

Stratified sampling technique was employed which presented the samples in levels with age variances. A total of seventy-five (75) male subjects were sampled for this study.

Study design

The study design is as follows;

Table 1: study design

Groups	Age	Observation
a	12-14	Biochemical test procedures 1 hour before onset of sleep and 1 hour after wakefulness
b	15-17	
c	18-20	
d	20-22	
e	22-24	
		N=15

The study duration was 42 days.

Inclusion criteria;

- ✓ Within the age-range
- ✓ Resident within the geographic region
- ✓ No family history of sleep or metabolic disorders

Exclusion criteria

- ✓ Exhibits some signs typical of psychophysiological disorders like generalized anxiety disorders (GAD) and attention deficit hyperactivity disorder (ADHD)
- ✓ Showed late night feeding behavior.

Biochemical tests

Test for cortisol

The Cortisol ELISA kit**,** ABNOVA trade name is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a polyclonal rabbit antibody directed against the cortisol molecule. The samples are dispensed in the coated wells and incubated with the enzyme conjugate (cortisol conjugated to horseradish peroxidase). During incubation endogenous cortisol of a sample competes with the enzyme conjugate for binding to the coated antibody. The unbound conjugate is removed by washing the wells. Subsequently, the substrate solution is added and the color development is stopped after a defined time. The intensity of the color formed is inversely proportional to the concentration of cortisol in the sample. The absorbance is measured at 450 nm with a microtiter plate reader.

Test for growth hormone

Enzyme-linked Immunosorbent Assay kit for growth hormone (GH) was used. Reagents, samples and standards were prepared. The procedure includes the addition of 100µL standard or sample to each well, 1 hour incubation at 37ºC; aspiration and addition of 100µL prepared Detection Reagent A; Incubation for 1 hour at 37ºC; aspiration and 3 times washing followed by the addition of 100µL prepared detection Reagent B. Incubate 30 minutes at 37ºC, aspirate and wash 5 times; addition of 90µL substrate solution. Incubation for 10-20 minutes at 37ºC, addition of 50µL stop solution. Read at 450nm.

Test for thyroxine

The Total Thyroxine (T4) ELISA is a competitive solid-phase enzyme-linked immunosorbent assay for the quantitative measurement of T4 concentration in human serum. The Total Thyroxine (T4) ELISA is comprised of three key components: Solid microwells pre-coated with anti-Total T4 antibody, Total T4 Calibrators, Total T4 Enzyme Concentrate (11X) comprised of horseradish peroxidase conjugated to T4 (T4-HRP). During the assay, the calibrator, control, or test specimen as well as the T4-HRP are added to the T4-antibody coated microwell. During the 60minute incubation, the T4 in the test specimen and in the T4-HRP compete to bind the antibody coated on the microwell surface. Unbound material is then removed by washing. In the next step, the TMB Substrate is added and the presence of the HRP bound to microwell surface is shown by the development of a blue color. The reaction is then terminated with the Stop Solution and the optical density (OD) is determined

using a spectrophotometer at 450/620-690 nm. The color intensity reflects the amount of T4-HRP bound to the microwell surface, and is inversely proportional to the amount of T4 in the control or test specimen. A standard curve can then be developed by plotting the Total T4 Calibrator concentrations on the x-axis against the relative OD values on the y-axis. The T4 concentration of each assay control or test specimen can then be interpolated from the standard curve.

Test for creatine kinase

Simple, direct and automation-ready procedures for measuring CK activity are very desirable. Creatine Kinase Assay Kit is based on enzyme coupled reactions in which creatine phosphate and ADP is converted to creatine and ATP by CK, the generated ATP is used to phosphorylate glucose by hexokinase to generate glucose-6-phosphate, which is then oxidized by NADP in the presence of glucose-6-phosphate dehydrogenase. The produced NADPH, measured at 340 nm, is proportionate to the CK activity in the sample.

Test for acetylcholinesterase

The Acetylcholinesterase Assay Kit is based on an improved Ellman method, in which thiocholine produced by the action of acetylcholinesterase forms a yellow color with 5,5'-dithiobis (2-nitrobenzoic acid). The intensity of the product color, measured at 412 nm, is proportionate to the enzyme activity in the sample.

Test for glutamate

Glutamate is quantitatively determined by ELISA. The competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The derivatized analyte concentrations in the standards, controls and samples and the solid phase bound analyte compete for a fixed number of antibody binding sites. When the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.

Statistical analysis

Statistical package for social sciences (IBM-SPSS®) version 22.0 was used for all statistical analysis. Data were presented in tables and graphs. Continuous variables were expressed as mean ± standard error of mean. Differences between groups were assessed by graph pad v.6.01 using one-way ANOVA and considering *p*-value less than 0.05 ($P<0.05$) as significant.

III. RESULTS

The following results were obtained from this study;

Table 2: time-dependent changes in stress hormones varies in different age groups

Age	Cortisol (µg/dL)		Growth hormone (ng/mL)		Thyroxine (µg/dL)	
	9pm	**6am**	**9pm**	**6am**	**9pm**	**6am**
12-14	25.12±0.03	21.24±1.13	2.30±1.21	2.56±0.22	0.73±0.71	2.93±1.10
15-17	23.15±0.12[a]	23.54±0.17[a]	1.93±0.25[a]	2.41±1.81	0.81±0.30	2.14±1.16
18-20	22.07±0.26[a]	24.13±0.42[a]	1.80±0.52[a]	1.31±0.20[a]	1.03±0.21	2.06±1.13
20-22	22.10±0.81[a]	25.18±0.24[a]	1.80±0.40[a]	1.15±0.13[a]	1.24±0.54[a]	1.78±1.16[a]
22-24	20.17±0.41[a*]	25.43±0.68[a*]	1.77±1.24[a*]	1.04±2.23[a*]	2.13±1.61[a*]	1.48±2.20[a*]
Total	**112.61**	**119.52**	**9.6**	**8.47**	**5.96**	**10.39**
Average	**22.52**	**23.90**	**1.92**	**1.70**	**1.19**	**2.10**

Key: [a]= Values are statistically different at $P<0.05$ compared to ages 12-14; *= most significant change in values compared to ages 12-14.

Age related response to the influence of stress signals on Cortisol, Growth Hormone and Thyroxine

There was a significant increase in the level of cortisol in all age range compared to ages 12-14. The most significant change was observed in ages 22-24 at 20.17±0.41 at 9pm and 25.43±0.68 at 6am. Growth hormone and thyroxine showed opposite relationship. There was a significant progressive decrease and increase in growth hormone and thyroxine in relation to age, respectively. The value for growth hormone at 22-24 years was 1.77±1.24 at 9pm and 1.04±2.23 at 6am while thyroxine was 2.13±1.61 at 9pm and 1.48±2.20 at 6am.

Table 3: time-dependent changes in muscle and a higher intellectual function and muscle function markers varies in different age groups.

Age	Creatine kinase (U/L)		Acetylcholinesterase (U/L)		Glutamate (mg/dL)	
	9pm	**6am**	**9pm**	**6am**	**9pm**	**6am**
12-14	36.11±0.03	34.21±0.09	40.20±1.82	39.13±0.17	45.34±0.03	40.32±0.71
15-17	38.24±0.42	37.71±0.30	41.26±0.22	48.50±1.24 [a]	46.62±0.05	46.75±0.52
18-20	56.32±0.08 [a]	51.00±0.26 [a]	50.71±1.43 [a]	56.88±0.30 [a]	48.86±0.10[a]	57.23±1.16 [a]
20-22	58.26±0.02 [a]	56.72±0.62 [a]	63.57±1.32 [a]	67.77±0.05 [a]	51.11±0.62 [a]	62.14±0.38 [a]
22-24	62.11±0.36 [a*]	60.77±0.03 [a*]	64.31±0.14 [a*]	69.21±0.11 [a*]	56.31±0.24 [a*]	68.84±0.36 [a*]
Total	**251.04**	**240.41**	**260.05**	**281.49**	**248.24**	**275.28**
Average	**50.21**	**48.10**	**52.01**	**56.30**	**49.65**	**55.10**

Key: [a]= Values are statistically different at P<0.05 compared to ages 12-14; *= most significant change in values compared to ages 12-14.

Age related response to the influence of stress signals on Creatine Kinase, Acetylcholinesterase and Glutamate.

Creatine kinase increased in units significantly from 18-20 years at 56.32±0.08 to 62.11±0.36 at 22-24 years, in both time of the day. Acetylcholinesterase and glutamate showed similar trend marked by a most significant increase at 9pm; 64.31±0.14, 6am; 69.21±0.11 and 9pm; 56.31±0.24 and 6am; 68.84±0.36, respectively.

IV. DISCUSSION

This study investigated the influence of stress signals on biochemical markers of physical activity and higher intellectual functions [10]. The body prepares for the day's stress by releasing stress hormones as early as 6 am from the *zona fasciculata* of the adrenal cortex [13-14]. Cortisol is the most predominant stress hormone [10-15] from the adrenal cortex that helps the body withstand the physical [11] and emotional exhaustion that accompanies our daily activities [12-13]. Some of the changes caused by the daily rhythmic secretion of cortisol include; promotion of glucose synthesis from non-glucose moieties, increased conservation of blood glucose, mobilization of all extrahepatic proteins and initiation of protein synthesis needed to withstand stress and sustained production of ATP through beta-oxidation of triacylglycerides [11-13]. Cortisol and growth hormone have similar effect on blood glucose regulation. They are diabetogenic hormones that affect learning and memory in distinctive ways [16]. Cortisol inhibits the functions of hippocampal neurons in learning and memory. Learning and memory are higher intellectual responses [15-16], both due to the interplay between neurons and neurocrine messengers [14]. The change observed in thyroxine level was more obvious in early ages with a progressive decrease as age advances [12]. Thyroxine is essential for nervous system growth and resistance to stress [10-12]. The increase in creatine kinase reveals the increased neuromuscular demand that accompanies ageing [13-16]. Acetylcholinesterase activity is suppressed during stress [16], a feature that may adversely affect learning and the influence is dependent on the degree of stress exposure [10]. This may be the likely reason for the reduced activity of acetylcholinesterase as shown in plasma in evening hours. Glutamate is excitatory in action, with excitotoxicity after prolonged exposure [11]. The build-up of glutamate with age during stress may be an adaptive response associated with learning and memory. Glutamate is the mediator for hippocampal NMDA receptors [16]. Previous exposure to stress may have influenced its rate of secretion in relation to age.

V. CONCLUSION

This study showed the biochemical changes in different age grades an hour before and after sleep periods. Although the fluctuations in the tested parameters were similar in most age groups, the variation was different in terms of significance. The change in stress and neuromuscular markers was directly related to the age of the samples as presented in this study.

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