EFFECT OF STRESS OF EXAMINATION ON SERUM CORTISOL LEVEL AND CD4 CELL COUNT IN MALE UNDERGRADUATES AT IGBINEDION UNIVERSITY, NIGERIA

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ABSTRACT

Aim: To assess the effect of stress of examination on serum cortisol level and CD4 cell count in young male students at Igbinedion University.

Methods: A cross sectional laboratory based analysis was adopted for this study. A total of 204 male undergraduate volunteers (age 22 ± 1.0 years, body mass index 23 ± 0.5 kg/m²) were randomly recruited for the study. Total white blood cell (TWBC) was determined using the Sysmex® Automated Hematology Analyzer. CD4 cell count was estimated using Partec cyflow counter, while serum cortisol level was determined by enzyme linked immuno sorbent assay technique.

Results: There was a significant increase (P < 0.05) in the serum cortisol level at Stage A (1st day of the semester) when compared with Stage B (midway in the semester) and Stage C (morning of the examination), while there was significant decrease (P < 0.05) in the TWBC count and CD4 cell count at Stage A when compared with Stage B and Stage C.

Conclusion: The stress of examination inhibits proliferation of CD4 cells with the elevation of serum cortisol as a possible mediator.

Keywords: Examination, Stress, Cortisol, CD4 cell.

INTRODUCTION

Neuroscientists believe that stress should be restricted to a condition where an environmental demand exceeds the natural regulatory capacity of the body. It is widely accepted that chronic stress can lead to down regulation of the immune function (Nakamura et. al., 1999). Cytokines are soluble communicators between components of the immune system and the brain (Maier and Watkin, 1998). Cytokines can be divided into pro-inflammatory cytokines such as interleukins (IL) – 2, interleukins (IL) – 6, tumor necrosis factor alpha (TNF α) and gamma interferon (IFN-γ) and...
anti inflammatory cytokines such as IL-4 and IL-10. (Maier and Watkin, 1998). Several stressors have been associated with a shift in cytokines production toward an anti-inflammatory pattern with cortisol as the proposed mediator (Elenkor and Chrousos, 2002). Cortisol elevation is one way the brain instructs the body to attempt to regain homeostasis by redistributing glucose to areas of the body that need it most (Heart and Brain) (Michael et al., 2001). It is generally accepted that T lymphocytes, particularly CD4 T lymphocytes, play a central role in immune response. Cellular immune deficiencies lead to increased susceptibility to infection from viruses, fungi, intracellular bacteria and protozoa (Abbas et. al., 2000). Prolong strenuous exercise has been shown to decrease the number of T lymphocytes and a more pronounced decrease in type 1 T cells, which might be linked to high plasma level of cortisol (Elenkor and Chrousos,2002) and adrenaline (Biselli et. al., 1993). Therefore, the present study was designed to assess the effect of stress of examination on serum cortisol level and CD4 cell count in young male students at Igbinedion University, Okada, Nigeria.

MATERIALS AND METHODS

Study Area
The study was carried out in the Department of Hematology, College of health sciences, Igbinedion University, Okada, Edo State, Nigeria between September 2012 to march 2013.

Study Design
A cross sectional laboratory based analysis was adopted for this study. A total of 204 male undergraduate volunteers (age 22 ± 1.0 years, body mass index 23 ± 0.5 kg/m²) were randomly recruited. The participants gave informed consent while the Board of ethical committee approved the study design. Subjects where advised not to eat, drink or exercise eight hours before blood sample collection. 5ml of venous blood was taken from the antecubital vein by venapuncture on the 1st day of the semester, midway in the semester and on the morning of the examination day. The blood was shared equally into an ethylene diamine tetra acetic acid (EDTA) container for total white blood cell count and CD4 cell estimation. The other portion was transferred into an anticoagulant free test tube, allowed to clot and subsequently centrifuged at 750xg for 15minutes to obtain the serum. The serum was immediately aliquoted into eppendorf tubes placed on ice and immediately stored at -80°C until serum cortisol was evaluated.

Exclusion Criteria
1. Hypertensive subjects and those with abnormal glucose level where excluded from the study.
2. Those diagnosed with viral, bacterial and parasitic infections where excluded.

EVALUATION OF PARAMETERS

Total White Blood Cell Estimation
Total white blood cell count was determined using the sysmex® Automated Hematology Analyzer Kx-21N, sysmex corporation, Kobe-Japan. It employs WBC detector block and WBC lyse reagent to measure WBC count as described by (Samuel et al., 2006).

CD4 Cell Estimation
CD4 cell count was estimated using Partec cyflow counter, Germany for the quantification of CD4 T lymphocytes as described (Partec cyflow counter 2010).

Serum Cortisol Estimation
Serum cortisol level was determined by enzyme linked immune sorbent assay technique. This test kit operates on the basis of competition between the hormone conjugate and the cortisol in the sample for a limited number of binding sites on the antibody coated plate. Twenty micro liters of standard or sample(s) was added per microplate. 200µl cortisol hormone conjugate was added to the standard or sample(s) and covered with a sealing tape. It was incubated at room temperature for 1 hour. The solution was discarded and microplates washed three times with 300µl of 1X wash solution. 100µl of Tetraethyl benzidine one step substrate was added to each microplate and incubated for 15 minutes at room temperature in the dark with gentle shaking. 100µl of stop solution was added to each microplate. The intensity of the color developed was measured at 450 nm wavelength using stat fax® 4700 micro strip reader. This method has been previously described by (Ehiaghe et. al., 2013).
Statistical Analysis
All numerical data were presented as mean ± standard deviation and analyzed using one way analysis of variance (ANOVA) and Turkey – Kramer Multiple comparison test using SPSS – 18.0 statistical program. P values < 0.05 were considered significant.

RESULTS

Table 1: Mean ± standard deviation of total white blood cell count, CD4 cell count and serum cortisol level at Stages A, B and C.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1st day of the semester (A)</th>
<th>Midway in the semester (B)</th>
<th>Morning of the examination (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total white blood cell count (cells/µl)</td>
<td>10500 ± 10</td>
<td>8000 ± 8³</td>
<td>6300 ± 8 PX</td>
</tr>
<tr>
<td>CD4 cell count cells/µl</td>
<td>1200 ± 5</td>
<td>850 ± 6³</td>
<td>650 ± 5 PX</td>
</tr>
<tr>
<td>Serum cortisol (ug/dl)</td>
<td>10 ± 0.02</td>
<td>50 ± 0.04³</td>
<td>110 ± 0.01 PX</td>
</tr>
</tbody>
</table>

There was a significant increase (P < 0.05) in the serum cortisol level at Stage A when compared with Stages B and C, while there was significant decrease (P < 0.05) in the TWBC count and CD4 cell count at Stage A when compared with Stages B and C.

KEY
S - Significant (P < 0.05) comparison between stage A and B
P - Significant (P < 0.05) comparison between stage A and C
X - Significant (P < 0.05) comparison between stage B and C
A - 1st day of the semester
B - Midway in the semester
C - Morning of the examination

DISCUSSION

The significant surge in serum cortisol level coupled with CD4 cell count reduction and Total white blood cells in Stage C as compared in Stages B and A (Table 1) could be attributed to the effect of prolonged serum cortisol secretion from the adrenal cortex which could be linked to stress associated with preparation for examination resulting in significant physiological changes. This is in accordance with the findings of Scott (2011), where cortisol is released in response to stressful events, coupled with the diversion of glucose from low priority organs in other to regain homeostasis. Cortisol prevents the proliferation of T cells by rendering the interleukin 2 producer T – cells unresponsive to interleukin – 1 and unable to produce the T cell growth factor (Palacious and Sugawera 1982) and inhibits immunoglobulin A and immunoglobulin M in serum (Posey et. al., 1978). The immune system is merely responding to the effect of stressor, during which most of the adaptation leads to greater fitness, if balance diet and resting are observed (Ehiaghe et. al., 2013). Long term exposure to cortisol damages cells in the brain (Hippocampus) which results in learning impairment and inability to retrieve already stored information (De Quervain et. al., 2000). Neuroscientists believe that stress should be restricted to condition where an environmental demand exceeds the natural regulatory capacity of the body, it is widely accepted that chronic stress can lead to down regulation of the immune function (Nakamura et. al., 1999). Moderately intense exercise stimulates the immune system and enhances resistance to infectious disease (Pedersen and Hoffman-Goetz, 2000; Pederson and Nieman, 1998). However, during strenuous activity there is often immuno suppression during the recovery period (Nieman, 1997). The post
exercise immune response is similar to that seen in both infection and inflammation, consisting of neutrophilia and lymphocytopenia (Tauler et al., 2004; Tauler et. al., 2006). The mechanism associated with these exercise induced immune changes includes oxidative stress as well as neuroendocrine factor such as catecholamine, growth hormone and cortisol (Pedersen and Nieman, 1998). Oxidative stress occurs when reactive oxygen species (ROS) and reactive nitrogen species (RNS) production overwhelm the antioxidant defenses system (Gomez et al., 2008). Lymphocytes appear to respond to oxidative stress challenges by increasing antioxidant enzymes may be the unique to exercise mode and duration. The immune suppression observed following exercise of a strenuous and prolonged nature has been linked to a decrease in circulating lymphocytes and a blunted natural killer cell activity (Pederson and Toft, 2000).

CONCLUSION
Stress of examination inhibits proliferation of CD4 cells with the elevation of serum cortisol as a possible mediator.

REFERENCES


