“Occupational Stress and Immune System Alterations: An Analysis of CD4 and CD8 Changes among Nurses in Private Medical Centres”

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ABSTRACT:
Stress may be defined as a state of mental anxiety caused by a challenging situation. In health care, employee workplace stress can have a negative impact on the quality of patient care, and significant effect on the occurrence of health problems leading to change the current working place and job, quit the profession, and interrupt relationship with co-workers. Studies demonstrated that job stressors could mediate the level of immunological biomarkers.

Aim & Objective: To analyse the occupational stress in nurses. i) To study the socio-demographic or job characteristics of nurses ii) To study the changes in immune biomarkers CD4 and CD8 iii) To study the changes in cytokines (IL-2, IL-6, IL-1β, TNF-α and INF-γ)

Methods: Male and Female Nurses of age group 25 to 60 were included. After administering self-reported questionnaires 55 subjects were selected for the study from various private medical centres, Coimbatore. Socio-demographic details and job characteristics were analysed and blood samples were collected for flow cytometry processing of CD4+ and CD8+ cells and cytokines were analysed through ELISA. Statistical analysis was done with SPSS version 22. Results: Overall the study population shows significant changes in CD8% and IL-6 levels, when compared with different levels of stress. CD4% showed numerical changes in nurses with moderate stress.

Conclusion: Nurses are sensitive to professional stress due to their intense daily activity. Since there is no specific marker for identification of stress, further studies can be carried out with a larger sample size to prove that CD8% can be used as a specific biomarker for stress analysis.

Introduction:
The term ‘Stress’ represents mental strain or anxiety caused by a problematic situation. The way we respond to stress, however, makes a big difference to our overall health. [1] In day-to-day life, it is necessary to have a small amount of stress which makes us stronger and more stable in daily activities. However, it may cause physical and mental health problems if it surpasses the limit. [2] Heavy workload, short deadlines, lack of appreciation and acknowledgement for work done, differences of opinion with colleagues or superiors, unpleasant work environment, job insecurity, and, most importantly, lack of social support can cause job stress regardless of the working field. Severe and chronic Job stress is a serious threat to the mental and physical well-being of any individual. [3] It may cause health issues like sleep disturbances, headaches, depression, anxiety, and burnout affecting mental peace and productivity at work.

A nurse’s job is no exception when it comes to Job stress, as it involves long working hours with late night shifts, dealing with life-and-death situations, and emotional demands by patients...
in pain and distress, and managing high workloads. The stress level in nursing jobs keeps increasing in spite of the use of technology in the medical field, higher medical costs, less number of nurses per shift, litigation, and, above all, the emerging and reemerging pandemic diseases. \[4\] Job stress in nurses affects their work performance, job satisfaction, punctuality, and physical and mental health. This can also lead to medical errors by nurses, which is a universal issue that can derail the entire patient care system. It was found that there is a valid connection between job stress, immunological inflammatory functions, and human diseases. \[5\] Studies demonstrated that job stress could bring about changes in immunological biomarkers such as CD4+, CD8+, and CD 57+ cells. In a study on Japanese blue-collar workers exposed to lead, an inverse correlation was seen between job stress and CD4 – T-helper cells. The interaction between psychological stress and immunological biomarkers was associated with several diseases like inflammatory diseases, cardiovascular diseases, infections and some autoimmune disorders. \[3\] Job dissatisfaction and disturbed mental health have been found in Japanese teachers.

Immunological parameter analysis studies have been performed in many occupations like Karate experts, Nuclear power plant workers, school teachers etc. \[7\] The main rationale of this study was that, to our cognizance, this was the first study that mainly analyzed the relationship between occupational stress and immunological changes in Nurses mainly CD4 and CD8 of southern Tamilnadu and thereby to identify the significant immune biomarker that plays a major role in stress.

**Materials and Methods**

**Subject selection.**

The research protocol was submitted to the Institutional Human Ethics Committee and it was approved (Ref No: IHEC-II/0058 /21). Nurses working at various private medical centres in and around Coimbatore were selected for the study. A total of 150 nurses were initially approached after repeated visits to their working hospitals. 92 of the nurses showed interest in participating in the study. An informed consent form was distributed to all the participants and the procedures were clearly explained. Both male and female nurses of the age group from 25 to 60 years were included and nurses with disease conditions like diabetes mellitus, immune system disorders, and autoimmune disorders were excluded from the study.

**Data collection – Questionnaire**

Data was collected through a self-administered questionnaire (Table:1). Basic demographic details and work-related characteristics such as job title, work shift, and working hours were investigated. After data collection, the questionnaires were scrutinized based on the inclusion and exclusion criteria, and out of 92 nurses, 55 were included in the study. Job stress was later scored on the basis of the perceived job stress scale with slight modifications.

Table: 1: Demographic and job characteristics

<table>
<thead>
<tr>
<th>Demographic and job details</th>
<th>N = 55</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
</tr>
<tr>
<td>Female</td>
<td>45</td>
</tr>
<tr>
<td>Age in years</td>
<td></td>
</tr>
<tr>
<td>25-35</td>
<td>16</td>
</tr>
<tr>
<td>36-45</td>
<td>10</td>
</tr>
<tr>
<td>46-55</td>
<td>17</td>
</tr>
<tr>
<td>56-60</td>
<td>12</td>
</tr>
<tr>
<td>Experience in years</td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>15</td>
</tr>
<tr>
<td>10-20</td>
<td>22</td>
</tr>
<tr>
<td>&gt;20</td>
<td>18</td>
</tr>
</tbody>
</table>
Sample collection

A blood sample was collected under standardised protocol from each of the participants in the nurse’s station and was immediately transferred to the laboratory for separation of serum. After centrifugation, the serum was stored in a deep freezer for analysis of cytokines. Anticoagulant EDTA-mixed tubes were used for the immunological parameter analysis.

Immunological and Inflammatory biomarkers measured:

The immunological biomarkers such as CD 4+ cells (T-helper cells) & CD 8+ cells (T-cytotoxic cells) and the inflammatory parameters such as Interleukin – 1β, Interleukin – 2, Interleukin – 6, Tumour necrosis factor – α, and Interferon –γ were the parameters whose levels were tested.

Immunooassays

The T-helper cells and T-cytotoxic cells (CD4+&CD8+ cells) were analysed by four colour flow cytometry (BD-FACSLyric™). This modern technique works on the principle of scattering of light and emission of fluorescence by a definite fluorescent probe labeled cells when they pass through a beam of lasers. Standard Lyse-no-wash procedure was used. 20μl of fluorochrome-conjugated monoclonal antibodies were added to 50μl of blood and incubated for 15 minutes at room temperature in the dark and then 450μl of 1X BD FACS lysis solution was added, mixed gently incubated at 15 minutes at room temperature. The sample was centrifuged at 1000 rpm for 5 minutes and the supernatant was discarded, then added 0.5ml of PBS was for analysis in a flow cyrometer and the results were expressed as cells/μl.

The inflammatory cytokines Interleukin–1β, Interleukin –2, Interleukin – 6, Tumor necrosis factor – α and Interferon –γ were assessed using the diacline solid phase sandwich ELISA for the in-vitro qualitative and quantitative determination. This is based on the principle that capture antibodies specific for the cytokines were coated to the wells of the microtiter plate. The binding of the cytokines present in the samples and known standards to the capture antibodies and subsequent binding of the biotinylated anti-cytokines secondary antibody to the analyte takes place during the same incubation period. The presence of excess unbound analyte and secondary antibody if any was removed. The HRP conjugate solution was then added to every well, and following incubation excess conjugate was removed by washing. A blue-colored complex will develop with the conjugate after addition of a chromogen substrate. The addition of acid to the substrate turns the colour to yellow. The concentration of the cytokines present in the samples and standards is directly proportional to the intensity of the product coloured complex. The absorbance of the colour complex is then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve.

Procedure: 100μl of each sample, control and zero were added in duplicate to appropriate number of wells following which 50μl of diluted biotinylated anti-cytokines was added to all wells and incubated in dark at room temperature for a given time period specific to each cytokine. The plates were washed thrice and 100μl of diluted streptavidin-HRP solution was added followed again by incubation in
the dark and washing thrice. The added 100μl of ready-to-use TMB substrate to all the wells and incubated in dark at room temperature. Finally, 100μl of H₂SO₄ stop reagent was added in all wells. The absorbance value of each well was read on a spectrophotometer using 450nm as the primary wavelength and 620nm as the reference wavelength.

**Statistical Analysis**

The study data were entered into the Microsoft Excel program. Statistical Package for Social Sciences (SPSS) Version 22, IBM Statistics, and USA was used for data analysis. The level of significance was set at 5% (p < 0.05 = Statistically Significant). ANOVA and Bonferroni test were used as statistically significant tests for comparison.

**Results:**

A total of 55 full-time working nurses were included in the study. The mean age was 46 years and 82% of the population was females with work experience of 10-20 years and 8 hours working per day. About 49.09% of the nurses reported more day shift work compared to night shift and 40% of them works in equal day and night shift work.

![Age-wise distribution of Nurses](image)

Figure: 1 Age-wise distribution of Nurses

Among the overall subjects, 89.09% of the nurses exhibited moderate levels of stress, 7.27% of the nurses exhibited a low stress level and 3.64% had high stress levels respectively. Statistical test for comparison was performed by ANOVA (P<0.05) and was found to be significant.

**Table: 2: Level of Stress in Nurses**

<table>
<thead>
<tr>
<th>STRESS LEVEL</th>
<th>NO OF SUBJECTS</th>
<th>STRESS SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low stress</td>
<td>4</td>
<td>10.75 ± 1.19 *</td>
</tr>
<tr>
<td>Moderate stress</td>
<td>49</td>
<td>16.64 ± 2.34 *</td>
</tr>
</tbody>
</table>
The results of immunological parameters CD4+ and CD8+ cells did not show any significant changes. Increased level of CD4% and CD8% was seen in nurses of the age group 44 to 55 years. No changes were seen in CD4:CD8 value. Table 3 shows the comparison between CD4%, and CD8% with all three levels of stress. The CD4% did not show any significant changes with the stress level, but CD8% levels were statistically significant on the comparison.

Table 3: Comparison of stress level and CD4%, CD8%

<table>
<thead>
<tr>
<th>STRESS LEVEL</th>
<th>CD4%</th>
<th>CD8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low stress</td>
<td>53.71 ± 5.29</td>
<td>24.32 ± 5.31*</td>
</tr>
<tr>
<td>Moderate stress</td>
<td>46.48 ± 8.54</td>
<td>27.07 ± 9.86*</td>
</tr>
<tr>
<td>High stress</td>
<td>53.66 ± 3.30</td>
<td>45.31 ± 3.50*</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± SD, statistically significant test for comparison was done by ANOVA followed by the Bonferroni test comparison between low stress vs. moderate & high stress, Moderate stress vs high-stress level –p-value < 0.05.

The ELISA tests for IL-6, TNFα, and IL-1β showed the following pattern of cytokine secretion by the subjects. About 47.27% of IL-6 and 12.73% of TNFα followed by 3.64% of IL-2, 1.64% of INFγ and no changes had been seen in IL-1β were seen. out of five inflammatory biomarkers only IL-6 shows significant changes when compared with moderate level of stress. The subjects with increased levels of CD8% secreted high levels of IL-6 and TNFα which shows a link between immunological and inflammatory markers. No significant changes were seen in low and high level of stress on comparison.

Table 4 Comparison of Stress level and Inflammatory biomarkers

<table>
<thead>
<tr>
<th>STRESS LEVEL</th>
<th>IL-2</th>
<th>IL-6</th>
<th>IL-1β</th>
<th>TNFα</th>
<th>INFγ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low stress</td>
<td>0</td>
<td>3.52 ± 4.21</td>
<td>0</td>
<td>4.07 ± 8.1</td>
<td>0</td>
</tr>
<tr>
<td>Moderate stress</td>
<td>0.59 ± 3.01</td>
<td>2.31 ± 3.77*</td>
<td>0</td>
<td>0.71 ± 2.48*</td>
<td>0.87 ± 6.2</td>
</tr>
<tr>
<td>High stress</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Discussion

Stress is the emotional and physiological response to unpleasant experiences. Stress related disorders and their ensuing disabilities are prevalent worldwide. Occupational stress results from a perceived imbalance between workplace pressure and coping abilities of the work. In nursing, work stress was first appraised by Menzies, who found that patient care, decision-making, taking
The cytokine IL-6 is produced by almost all immunological and stromal cells, such as B lymphocytes, T lymphocytes, macrophages, dendritic cells, monocytes, mast cells, fibroblasts, and endothelial cells. IL-1 and TNF are the main inducers of IL-6 expression. Its secretion may also be influenced by several other factors, including Toll-like receptors, prostaglandins, adipokines, stress response, and other cytokines. Through a variety of signal transduction pathways, IL-6 unites with its receptor, IL-6R, to generate a transmembrane and soluble form. This complex then attaches to gp130, triggering the classical pathway's gene expression. Additionally, activated are the Janus kinase/signal transducers and activators of transcription, Rat sarcoma virus - rapidly accelerated fibro carcinoma (JAK-STAT, RAS-RAF) and other pathways, which support cellular differentiation, proliferation, oxidative stress, and immunological control. [11]

Sarah et al, in their study on CD8+ T cells cytokines responses to stress targets, the role of the T-cells on cytokine level after being exposed to stress and concluded that T cell may provide important modulatory functions on cytokine production in response to mental stress. [14] Finally from this study, it was observed that job stress has a strong relation to CD8% thereby to IL-6. Follow-up analysis supports the hypothesis that immunological inflammatory changes concerning occupational stress. These immune alterations have materiality for proneness to diseases

Conclusion.

From our study, we conclude that job stress can alter the level of immunological biomarkers, and the implementation of coping strategies and awareness of job stress-mediated health issues among nurses may reduce the risk of susceptibility to diseases. Future studies with different populations and more subjects may create a useful database for the benefit of healthcare workers.

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Conflict of interest statement: NO
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Bibliography:

16. M. Manzaneque ,1 Francisca M. Vera ,1 Gabriel A. Carranque,2Francisco M. Rodriguez-Peña ,3 Federico Navajas,3 and Maria J. Blanca 1 Immunological Modulation in Long-Term Karate Practitioners Hindawi Evidence-Based Complementary and


