

A Hospital-Based Assessment of the Effect of Perceived Stress on the Leukocyte Profile of Healthy Young Adults between the 18 to 21 Years of Age Group

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Abstract

Aim: The present study was planned and carried out with the aim to study the effect of perceived stress on the leucocyte profile of healthy, young adults.

Methods: A prospective study was conducted in the Department of Physiology, Nalanda Medical College, Patna, Bihar, India, on 1st Prof MBBS students, aged 18 to 21 years, before and during their 1st semester examination. The study procedure and objectives of the study were explained to the students. Participation in the study was solely on a voluntary basis. All 50 students gave written informed consent to take part in the study.

Results: In response to how frequently they felt nervous or stressed during the last month, 20 subjects responded "Sometimes" and an equal percentage responded "Often/Always". Based on the PSS scores, the students were grouped as "Not stressed" (PSS score 1-10), "Mildly stressed" (PSS score 11-20), "Moderately stressed" (PSS score 21-30) and "Severely stressed" (PSS score 31-40). None of the study participants had PSS score greater than 30. 27 subjects were found to be mildly stressed and 18 students were moderately stressed. For each group of study participants, the average N:L ratio was calculated. The Pearson correlation coefficient (r) was calculated to study the relation between the PSS score and the relative percentages of the different leukocytes as well as the N:L ratio. A positive correlation was found between the PSS score and percentage of neutrophils, monocytes and N:L ratio. A negative correlation was found between PSS score and percentage of lymphocytes, eosinophils and basophils.

Conclusion: Examinations in medical school are stressful enough to produce changes in heart rate, blood pressure and differential leucocytes counts although all the students were in good health status.

Keywords: Stress, Examination, DLC, Blood Pressure, Heart Rate.

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Introduction

The term stress was coined by the endocrinologist Hans Selye [1] and is defined as any change in the environment that changes or threatens to change an optimal existing state. [2] Stress causes an imbalance of the parasympathetic and sympathetic nervous system due to psychic stimuli which lead to disturbance of homeostasis in the body. [3] Stress has been reported to influence the development and progression of atherosclerosis in general population [4] and may explain part of the differential cardiovascular and cerebrovascular mortality rates. [5] Physiological studies have shown that stress can affect the blood cell parameters. [6]

Perceived stress refers to an individuals' perception regarding the level or amount of stress that he/she is experiencing at a particular point of time or during a given time period. Therefore, it includes feelings regarding the unpredictable nature of events in one's life, the frequent irritations and various changes occurring in one's life as well as a person's confidence in his/her ability to deal with such stressful situations. Perceived stress is, therefore, a reflection of an individuals' interaction with his/her environment and depends on the individuals' own perception. The Perceived Stress Scale (PSS) [7] was developed by Sheldon Cohen and his colleagues in 1983 to measure the degree to which an individual regards events/situations in his/her life as stressful. Before the development of

the PSS, the measurement of stress was primarily based on objective measures, such as the frequency of occurrence of specific stressors e.g. chronic illness, loss of a family member etc.

There is an enormous amount of literature on psychological stress and cardiovascular disease. Cardiovascular diseases claim the dominant trajectory involves atherosclerosis, a chronic inflammatory process of lipid-rich lesion growth in the vascular wall that can cause life-threatening myocardial infarction (MI). Leukocytes - white blood cells - are important participants at the various stages of cardiovascular disease progression and complication. Atherosclerosis is the pathology that leads to myocardial infarction and stroke. Atherosclerosis is a chronic inflammatory disease driven by lipids, specifically low density lipoproteins (LDL) and leukocytes. Lifestyle, age, hereditary factors, and comorbidities disturb immune, digestive, endocrine, circulatory, and nervous systems, thereby altering immune function, metabolism, and many other processes, while eliciting inflammation, hypercholesterolemia, and hypertension. [8] Leukocyte profiles are altered by stress and can be directly related to stress hormone levels. Specifically, the changes brought on by stress are increase in the number of neutrophils (neutrophilia) and decrease in lymphocyte count (lymphopenia or lymphocytopenia). Moreover, since the number of neutrophils and lymphocytes are affected by stress in opposite directions, researchers have often considered the ratio of one to the other, that is, the relative proportion of neutrophils to lymphocytes (N:L ratio) in mammals as a composite measure of the stress response. This ratio, as read from standard blood smears made before and after a stressful event, is positively related to the magnitude of the stressor and to the circulating glucocorticoids. [9] Hence the present study was planned and carried out with the aim to study the effect of perceived stress on the leucocyte profile of healthy, young adults.

Material & Methods

A prospective study was conducted in the Department of Physiology, Nalanda Medical College, Patna, Bihar, India. 1st Prof MBBS students, aged 18 to 21 years, were recruited for the study. The study procedure was carried before and during their 1st semester examination. The study procedure and objectives of the study were explained to the students. Participation in the study

was solely on a voluntary basis. All 50 students gave written informed consent to take part in the study.

The study tools were:

1. Perceived stress scale - used to measure perceived stress among students.
2. Differential leukocyte count - to study the leukocyte profile of the students.

Perceived Stress Scale (PSS):

The study participants completed the 10-item Perceived Stress Scale (PSS-10; Cohen and Williamson, 1988), which measured the degree to which the individuals perceived their daily life during the past month as stressful. PSS-10 consists of 10 questions, with responses varying from 0 to 4 for each item and ranging from Never, Almost never, Sometimes, Fairly often and Very often respectively, on the basis of occurrence during one month prior to the survey. The possible range of scores varies from 0-40. The score on the positive items (question no. 4,5,7,8) are reversed (e.g. 0=4, 1=3, 2=2, 3=1, 4=0) and then the scores of all the ten items are added to yield a single score. Higher scores on the PSS-10 represent higher levels of perceived stress.

Differential Leucocyte Count (DLC):

It was performed by staining a peripheral blood smear with Leishman's stain and 100 leucocytes were counted using the oil immersion objective.

Volunteers were selected after taking detailed clinical history. The subjects with any pre existing disease, or any medication, were excluded from the study. Those subjects with any addiction to tobacco or alcohol were also excluded from the study.

The study was carried out in the department of Physiology, NMC with informed consent. Three months prior to the semester examination (the students being unaware of the forthcoming examination) were subjected to clinical check-up (Blood pressure and Heart rate) and estimation of DLC by staining the peripheral blood smear with Leishman's stain. Cells were counted using Compound Microscope and similar procedure was repeated on the day of semester examination (in between hematology practical and viva-voice).

Obtained data was analyzed and compared with pre-examination results

Results

Table 1: Sample response frequencies for the 10-item PSS survey

Question	Never	Almost never (Rarely)	Sometimes	Fairly Often	Very often (Always)
In the last month, how often have you been upset because of something that happened unexpectedly?	8	18	10	8	6
1. In the last month, how often have you felt that you were unable to control the important things in your life?	4	9	16	14	7
3. In the last month, how often have you felt nervous and "stressed"?	3	7	20	12	8
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	0	5	15	18	12
5. In the last month, how often have you felt that things were going your way?	7	11	12	15	5
6. In the last month, how often have you found that you could not cope with all the things that you had to do?	4	9	12	17	8
7. In the last month, how often have you been able to control irritations in your life?	7	10	11	15	7
8. In the last month, how often have you felt that you were on top of things?	8	9	19	11	3
9. In the last month, how often have you been angered because of things that were outside of your control?	8	12	17	9	4
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	9	11	16	8	6

In response to how frequently they felt nervous or stressed during the last month, 20 subjects responded "Sometimes" and an equal percentage responded "Often/Always".

Table 2: Mean PSS scores and N:L ratios of study population

PSS score	Classified as	Mean PSS	SD	No.	N:L ratio
1-10	Not stressed	8.62	0.55	5	1.42
11-20	Mild stress	16.32	4.36	27	1.55
21-30	Moderate stress	26.34	3.14	18	1.70

Based on the PSS scores, the students were grouped as "Not stressed" (PSS score 1-10), "Mildly stressed" (PSS score 11-20), "Moderately stressed" (PSS score 21-30) and "Severely stressed" (PSS score 31-40). None of the study participants had PSS score greater than 30. 27

subjects were found to be mildly stressed and 18 students were moderately stressed. For each group of study participants, the average N:L ratio was calculated. It was seen that the moderately stressed group had higher N:L ratio compared to the mildly stressed and not stressed groups.

Table 3: Correlation between levels of perceived stress and leucocyte counts

Correlation between PSS and	Correlation coefficient (r)
Neutrophil percentage	0.34
Lymphocyte percentage	-0.30
Eosinophil percentage	-0.16
Monocyte percentage	0.25
Basophil percentage	-0.26
N:L ratio	0.34

The Pearson correlation coefficient (r) was calculated to study the relation between the PSS score and the relative percentages of the different leukocytes as well as the N:L ratio. A positive correlation was found between the PSS score and

percentage of neutrophils, monocytes and N:L ratio. A negative correlation was found between PSS score and percentage of lymphocytes, eosinophils and basophils.

Discussion

Medical education course curriculum, examination pattern, fear of failure, inability to cope with first exposure to a very different system of education and competition among peer are extremely stressful condition for students. [10-12] Academic stress in medical school has not only an immediate impact on the academic performance, but can also lead to cynicism in the form of decreased empathy and humanitarianism. [13] Stress causes an imbalance of the parasympathetic and sympathetic nervous system due to psychic stimuli which lead to disturbance of homeostasis in the body. [14] Stress has been reported to influence the development and progression of atherosclerosis in general population [15] and may explain part of the differential cardiovascular and cerebrovascular mortality rates. [16] Physiological studies have shown that stress can affect the blood cell parameters. [17]

Stress and disease arise from a lack of integration of the various physiological systems with the holistic "inner intelligence" of the body. [18] This may result in loss of homeostasis in the CVS that could be expressed as higher BP. Raised blood pressure is associated with increased risk of cardiovascular disease. In the present study an increased blood pressure was found in subjects with higher PSS score.

Numerous studies have shown that stress and stress hormones induce significant changes in absolute number and relative proportions of leukocytes in blood. [19,20] In the present study, a positive correlation was found between the PSS score and percentage of neutrophils, monocytes and N:L ratio. A negative correlation was found between PSS score and percentage of lymphocytes, eosinophils and basophils.

The findings of present study are similar to the study done by Sharma N et al. [21] Sharma N et al. studied the effect of examination stress on hematological and hemodynamic parameters in Students and reported higher neutrophil count during examination

Similarly Qureshi F et al. also found higher neutrophil count and lower lymphocyte count during the examination period as compared to the pre examination levels. [22]

A higher N:L ratio during the stress may be attributed to the action of various hormones such as glucocorticoids and catecholamines. [23,24]

Conclusion

Examinations in medical college are stressful enough to produce changes in heart rate, blood pressure and differential leukocytes counts which may affect their health and day to day activities. Results of the present study showed significantly

higher N:L ratio, in subjects with higher stress scores. Thus differential leukocyte count (DLC) can be used for assessment of stress. DLC is an easy and inexpensive test to perform. This test when combined with biochemical parameters like serum cortisol can provide better insight to study stress response. Better assessment of stress response can help us devise better coping strategies for a healthier living.

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