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Original Research Article

A Hospital-Based Study to Determine the Changes Brought on by Perceived Stress in the Leukocyte Profile of Young Adults

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Abstract

Aim: The present study was carried out to determine the changes brought on by perceived stress in the leukocyte profile of young adults.

Methods: The study design was approved by the Institutional Ethical Committee , Patna Medical College, Patna, Bihar, India. The study population consisted of the students' attending classes in the department of Physiology, Patna Medical College, patna, Bihar, India for one year. The study procedure and objectives of the study were explained to the students. Participation in the study was solely on a voluntary basis. Out of the 70 students, 50 students gave written informed consent to take part in the study giving a response rate of about 71%.

Results: The mean PSS score of the study participants was found to be 19.14±6.36. In response to how frequently they felt nervous or stressed during the last month, 36% (18 nos.) 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. 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: Several researchers have put forward the concept of use of DLC parameters such as neutrophilia, lyphocytopenia or N:L ratio as an adjunct to the measurement of adrenal glucocorticoids, especially Cortisol, for studying the stress response. There is ample evidence which suggests that there is a close relationship between the responses of the leukocytes and the adrenal hormones to stress.

Keywords: perceived stress, DLC, neutrophil, lymphocyte, N:L ratio.

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] 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. Individuals exposed to similar life situations may experience different levels of stress, depending on their personality, coping strategies and support system. 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) [3] 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. This form of assessment, however, does not take into consideration the fact that the same stressor may be interpreted differently by different persons, resulting in a novel subjective experience for each individual. The PSS was developed by Cohen and his colleagues to add the subjective component to the assessment of stress. The original PSS consists of 14 items that are purported to form a unidimensional scale of global perceived stress. Although scores on the 14-item PSS tend to exhibit good reliability estimates across the literature, four of the items tend to perform poorly when evaluated using exploratory factor analysis. [4]

Stress causes an imbalance of the parasympathetic and sympathetic nervous system due to psychic stimuli which lead to disturbance of homeostasis in the body. [5] Stress has been reported to influence the development and progression of atherosclerosis in general population [6] and may explain part of the differential cardiovascular and cerebrovascular mortality rates. [7] Physiological studies have shown that stress can affect the blood cell parameters. [8]

The present study was carried out to determine the changes brought on by perceived stress in the leukocyte profile of young adults.

Materials and Methods

The study design was approved by the Institutional Ethical Committee, Patna Medical College, Patna, Bihar, India. The study population consisted of the students attending classes in the department of Physiology, Patna Medical College, Patna, Bihar for one year. The study procedure and objectives of the study were explained to the students. Participation in the study was solely on a voluntary basis. Out of the 70 students, 50 students gave written informed consent to take part in the study giving a response rate of about 71%.

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.

Results

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

Question	Never	Rarely	Sometimes	Often	Always
1. In the last month, how often have you been upsetbecause of something that happened unexpectedly?	8	15	7	2	5
2. In the last month, how often have you felt that youwere unable to control the important things in your life?	2	7	12	12	4
3. In the last month, how often have you felt nervousand "stressed"?	3	6	18	9	5
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	0	2	7	11	17
5. In the last month, how often have you felt thatthings were going your way?	6	8	8	11	4
6. In the last month, how often have you found that you could not cope with all the things that you had todo?	3	8	9	13	4
7. In the last month, how often have you been able tocontrol irritations in your life?	6	6	6	13	6
8. In the last month, how often have you felt that youwere on top of things?	7	8	15	6	1
9. In the last month, how often have you been angeredbecause of things that were outside of your control?	6	9	12	7	3
10. In the last month, how often have you felt difficulties were piling up so high that you could notovercome them?	9	8	10	6	4

The mean PSS score of the study participants was found to be 19.14±6.36. In response to how frequently they felt nervous or stressed during the last month, 36% (18 nos.) 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	9.67	0.58	3	1.47
11-20	Mild stress	15.63	3.34	19	1.62
21-30	Moderate stress	25.47	3.29	15	1.75

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. 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	0.36
Lymphocyte percentage	-0.32
Eosinophil percentage	-0.15
Monocyte percentage	0.21
Basophil percentage	-0.23
N:L ratio	0.38

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. [9,10] 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. [11] Stress during examination among medical students is a well-known phenomenon encountered worldwide, there are other possible stressors to which medical students may be exposed. [12,13] Physiological studies have shown that stress from any source can influence on the endocrine, hemopoietic and immune system. Cytokine and cortisol seems to play an important role in the communication between these systems. [14,15]

From the perspective of Maharishi Vedic Medicine, stress and disease arise from a lack of integration of the various physiological systems with the holistic "inner intelligence" of the body. [16] This may result in loss of homeostasis in the CVS that could be expressed as higher BP, or atherosclerosis (in long standing condition). Evidence indicates that chronic psychosocial stress induces excessive adrenergic activation and sympathetic hyper responsively, leading to carotid atherosclerosis. [17,18]

In relation to the hematopoietic system, changes that have been reported by previous studies include increase in the numbers of neutrophils and platelets and decrease in the numbers of lymphocytes, eosinophils and monocytes. Earlier studies have used examination as a stressor and have compared the absolute count of leucocytes in the pre and post examination period. Higher neutrophil count during or

post-examination has been reported by Neena et al. [19] , Qureshi et al. [20] as well as Mantur and Murthy. [21] Lower counts of lymphocytes, eosinophils and basophils during or post- examination compared to pre-examination counts have been reported by Neena et al. [19] and Qureshi et al. [20] The findings of the present study are in conformity with those of the previous studies.

In relation to the monocyte count, Neena et al. [19] and Qureshi et al. [20] have reported that counts are lower during or post-examination whereas Mantur and Murthy21 found higher counts during examination stress. The present study found higher monocyte numbers in participants with higher levels of perceived stress; this finding conforms to that of Mantur and Murthy. [21] Numerous studies have shown that stress and stress hormones induce significant changes in absolute number and relative proportions of leukocytes in blood. Dhabhar et al. [22,23] were the first to propose that stress induced changes in blood leukocyte distribution may represent an adaptive response. They suggested that acute stress induced changes in blood leukocyte number represent a redistribution of leukocytes from blood to other organs such as the skin and lining of gastrointestinal tract and the genito-urinary tract and draining sentinel lymph nodes. [24] They hypothesised that such a reduction may enhance immune function in those compartments to which immune cells travel during stress.

The catecholamines, epinephrine and norepinephrine, and adrenal glucocorticoid hormones have been identified as the major endocrine mediators of stress induced changes in leukocyte distribution. [25-27] In response to glucocorticoids, circulating lymphocytes adhere to the endothelial cells that line the walls of the blood vessels and subsequently, undergo transmigration from the circulation into other tissues e.g. lymph nodes, spleen, bone marrow and skin, where they are sequestered. [28-30] This exodus of lymphocytes from the blood causes a significant reduction in their circulating numbers. In contrast, glucocorticoids also stimulate an influx of neutrophils into the blood from the bone marrow and decrease the efflux of neutrophils from the blood to the other compartments. [31] These changes are thought to ensure that the different types of cells are routed to where they are needed during the stress response [32] and result in an increase in N:L ratio that is proportional to the level of glucocorticoid release. Different types of stressors may affect the hematological parameters e.g. N:L ratio may increase after strenous exercise in humans. [33,34]

Conclusion

Several researchers have put forward the concept of use of DLC parameters such as neutrophilia, lyphocytopenia or N:L ratio as an adjunct to the measurement of adrenal glucocorticoids, especially Cortisol, for studying the stress response. There is ample evidence which suggests that there is a close relationship between the responses of the leukocytes and the adrenal hormones to stress. Advantages of studying the leukocyte response by doing a DLC include low cost and ease of performing the test in a small laboratory with minimal infrastructure. Thus, studying the leukocyte profile by performing a differential leukocyte count can provide a reliable method to assess the stress response. In view of the limitations of the present study, a longitudinal study with more subjects and examination of DLC smears before and after a stressful event, followed by correlation with PSS scores, along with measurement of serum cortisol levels shall be more informative and helpful.

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