Effect of Perceived Stress on the Differential Leucocyte Count among Young Adults

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Abstract

Exposure to any form of stress causes an increase in ACTH secretion by the anterior pituitary, followed by increased secretion of Cortisol, which decreases eosinophils, basophils and lymphocytes in blood and increases the number of neutrophils.With the above background, the present study was carried out to determine the changes brought on by perceived stress in the leukocyte profile of young adults.37 healthy, young adults participated in the study. Perceived stress was measured using the 10-item Perceived Stress Scale (PSS-10) and leukocyte profile was studied by performing a differential leukocyte count.A positive correlation was found between 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.Stress identification and management is an important component in the welfare of young adults. Differential count of leukocytes can provide a reliable method to study the response 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)[(4)] 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. [(5)] As a result, the PSS is commonly implemented using the 10-item form. [(6)] Cohen et al. (1988) further reduced the PSS to a four item form for quick measurements; however, scores on the 4-item PSS tend to exhibit lower reliability estimates

than researchers would like. [5]

The leukocytes, also called white blood cells, are the mobile units of the body's protective system. [7] Morphologically, there are five types of leucocytes in blood: Neutrophils, Eosinophils, Basophils, Lymphocytes and Monocytes. The first three types of cells, the polymorphonuclear cells, all have a granular appearance and for this reason are called granulocytes or, in clinical terminology, "polys" because of multiple nuclei. [7] Normally, human blood contains 4,000-11,000 white blood cells per microlitre. Of these, the granulocytes (Polymorphonuclear leucocytes, PMNs) are the most numerous. Young granulocytes have horse-shoe shaped nuclei that become multilobed as the cells grow older. Most of them contain neutrophilic granules (Neutrophils), but a few contain granules that stain with acidic dyes (Eosinophils) and some have basophilic granules (Basophils). The other two cell types found normally in peripheral blood are Lymphocytes, which have large, round nuclei and scanty cytoplasm, and Monocytes, which have abundant agranular cytoplasm and kidney-shaped nuclei. Of the total white blood cells, the normal percentages of the different types of leucocytes are as follows: Neutrophils (50-70%), Eosinophils (1-4%), Basophils (0.4%), Lymphocytes (20-40%) and Monocytes (2-8%). [2]

Leucocyte 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. [8]

Aims and Objectives

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. The study objectives were:

1. To assess the level of perceived stress of the study subjects

2. To determine the relation between the level of perceived stress and the relative percentages of the different leukocytes (neutrophils, eosinophils, basophils, lymphocytes and monocytes)

3. To determine the relation between the level of perceived stress and the Neutrophil : Lymphocyte (N:L) ratio

Materials and Methods

The study design was approved by the Institutional Ethical Committee, Gauhati Medical College, Guwahati. The study population consisted of the first year D.Pharm students attending classes in the department of Physiology,Gauhati Medical College. 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 60 students,37 students gave written informed consent to take part in the study giving a response rate of about 62%. The study was a cross-sectional observational 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.

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, 36.84% (14 nos.) responded "Sometimes" and an equal percentage responded "Often/Always". (Table 1)

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 upset because of something that happened unexpectedly?	8	15	7	2	5
2.In the last month, how often have you felt that you were unable to control the important things in your life?	2	7	12	12	4
3.In the last month, how often have you felt nervous and "stressed"?	3	6	14	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 that things 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 to do?	3	8	9	13	4
7.In the last month, how often have you been able to control irritations in your life?	6	6	6	13	6
8.In the last month, how often have you felt that you were on top of things?	7	8	15	6	1
9.In the last month, how often have you been angered because 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 not overcome them?	9	8	10	6	4

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 (Table 2). It was seen that the moderately stressed group had higher N:L ratio compared to the mildly stressed and not stressed groups.

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

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 (Table 3). 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.

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

Discussion

Various types of stressors may have an impact on the functioning of the different systems of the body. 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. The present study,on the other hand,has measured the perceived stress level and correlated the PSS score with relative percentages of the different leucocytes.

Higher neutrophil count during or post-examination has been reported by Neena et al.[9] , Qureshi et al. [10] as well as Mantur and Murthy.[11] Lower counts of

lymphocytes, eosinophils and basophils during or post-examination compared to pre-examination counts have been reported by Neena et al. [9] and Qureshi et al. [10] The findings of the present study are in conformity with those of the previous studies.

In relation to the monocyte count, Neena et al. [9] and Qureshi et al. [10] have reported that counts are lower during or post-examination whereas Mantur and Murthy [11] 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. [11]

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. [12,13] 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. [14] 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 adrenalglucocorticoid hormones have been identified as the major endocrine mediators of stress induced changes in leukocyte distribution.[15-20] 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.[21-23]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.[24] These changes are thought to ensure that the different types of cells are routed to where they are needed during the stress response[20,25]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. [26]

Limitations of leucocyteprofiles: The relative percentages of the different leukocytes can give us an idea regarding the amount of stress being experienced by an individual relative to others. However, the DLC only provides information regarding the percentages of WBCs currently in circulation; nothing can be said with much certainty about the number of neutrophils or lymphocytes that are being held in reserve in the other body compartments or how many of these cells would be released in response to a stressor. The results of DLC from a single smear, therefore, does not tell us much regarding an individuals' immune capacity. Performing the differential count on two separate occasions, one before and the second after a stressor event, can give us an idea regarding an individuals' ability to mount an immune response. [8]

Also, there may be some confusion in differentiating leukocyte responses to stress from those due to inflammation or infection, as in both situations, leukocyte profiles showneutrophilia and lymphocytopenia. However, infections, in addition, cause increases in monocyte percentage, as they also help in phagocytosis of foreign particles [27-29] and general increases in total WBC count.[27,30-32] Further, according to Jain,[27] reduction in relative eosinophil numbers is more likely to occur in response to stress rather than as a response to disease. Therefore, by taking into account the relative percentages of the different leukocytes and studying the leukocyte profile as a whole, we can differentiate the stress response from the effect of disease.

Limitations of study: The present study is a cross-sectional type of observational study. Only a single DLC smear was prepared and examined in a small number of subjects. Also serum Cortisol level was not estimated.

Conclusion

Several researchers have put forward the concept

of use of DLC parameters such as neutrophilia, lyphocytopenia or N:L ratio as an adjunct tothe 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. [8]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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