ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.pharmascope.org/ijrps

Distribution of leukocyte subpopulation among students threatened by failure

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Article History:

Received on: 19 Apr 2020
Revised on: 20 May 2020
Accepted on: 19 Jun 2020

Keywords:

Stress,
anxiety,
depression,
immunity,
neuro-immunology,
leukocytes

ABSTRACT

Psychoneuroimmunology or the study of the relationships between the brain and the immune system is an area of research that has experienced significant development over the decade. Stress does not appear without consequences on the state of health, the role of fears, emotions and significant constraints in the appearance of organic and mental diseases. In this research, we studied the effect of stress and anxiety during exams at the end of the academic year (2018/2019) on the distribution of leukocyte subpopulations and the immune system, questionnaires has been completed by student volunteers, to estimate the anxio-depressive comorbidities through the (HADS) test during and outside exams, and in the same time we asked them for a blood sample the next morning day to carry out some biological assays (CBC). We also found that stress during exams caused a change in the distribution of different types of white blood cells, a total decrease in white blood cell counts with neutropenia and lymphopenia were found in students during exams compared to controls, and an increase in monocyte and other types of polymorphonuclear levels in students during exams compared to controls. Other tests measuring the effects of stress on specific functions of the immune system can be used.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i3.2553

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INTRODUCTION

Maintaining the homeostasis of the living organism is ensured in mammals by three major communication and integration systems: central nervous system (CNS) and autonomic nervous system (ANS), endocrine system (SE) and immune system (IS).The operation of these three systems has long been considered independent, but that numerous recent data show that they communicate with each other in a way that is both multi directional and very precise. This communication is achieved through the production of common mediators (hormones, cytokines, neuromediators, neuropeptides) and the presence of receptors specific to each of them (Blalock, 1984). Because of the community of pathways they take and the mediators and receptors they use, stresses of a different nature (physical, psychological, infectious or toxic) can have converging effects. Each system is subject to a set of activation factors, but also very fine regulation (feedback loops or feedback). A deficit at this level can have serious consequences. Thus, for example, the immune system assumes a role of surveillance and intervention, including in the brain. An excessive response from glial cells (cells of the nervous system involved in immune reactions) can have deleterious effects on neurons. Similarly, at the level of the SE, the feedback loops if they no longer fulfill their role (in particular that which involves corticosteroids), they could favor the establishment of several psychiatric pathologies, among which anxiety, depression and other mood disorders (Brochier and Olié, 1993). In this perspective and considering the multiple psycho-neuro-immuno-endocrine interactions, our work presents how situations of acute stress, or as an example the period of exams at the end of the year affecting the function of the immune system in students.

PATIENTS AND METHODS

Recruitment of subjects

This study was carried out on 30 students of the Faculty of Natural and Life Sciences of the Mentouri Constantine University, during the exam period of the 2018/2019 academic year. The students were selected according to the average of the first semester, the average of which was less than 10/20 and therefore threatened with failure if they did not have an average greater than or equal to 11/20 or second semester, this makes them more anxious and nervous during the exam period.

The same group of students was tested in a period outside the exam period. In this research, we studied the effect of stress and anxiety during exams at the end of the year on the distribution of leukocyte subpopulations and the immune system, we distributed questionnaires to be completed by student volunteers, to estimate the anxiety-depressive comorbidities through the HADS test (Hospital Anxiety and Depression Scale) during and outside exams, and at the same time we asked them for a blood sample the next morning to carry out some biological assays (CBC).

Statistical analysis of results

The results are presented as an average plus or minus the standard deviation ($m \pm s$) and illustrated by tables. The statistical analysis was carried out using MINITAB statistical software where we used the generalized linear model to make an overall statistical comparison of the sample means (ANOVA test). This module automatically gives us the result of the comparison test of means with respect to the control average (Dunett test).

RESULTS AND DISCUSSION

We note in students outside exams 13 subjects who do not have a depressive state, 2 subjects at the start of depressive state on the other hand, in students during exams we note that only one subject who does not have depressive state and 7 subjects at the start of depressive state and 7 subjects in highly depressed state (Table 1). The results of leucocytes variations and leucocytes subpopulations were recorded in Table 2.

Occurrence of mood disorders (depression and anxiety) in students not taking exams and during exams. In our study we found that there is a close relationship between the degree of anxiety and the emergence of depressive disorders (Table 1) and the threat of failure in the examinations of the end of the year among the students, or we recorded on the scale of HADs distributed and filled by students during and outside exams, very high values of anxiety in students threat of failure compared to students outside exams, the same for the emergence depressive disorders where we recorded 13 cases no depression among students without exams among the 15 cases studied compared to 14 cases between the onset of depressive state and highly depressive state among the 15 cases studied by students during exams and threat of failure Some areas of study appear to be associated with more psychological distress. Several studies have addressed, among other things, the psychological distress present in health science students, particularly the distress of medical students (Dyrbye *et al.*, 2006).

According to the literature review by Dyrbye *et al.,*(2006), medical students are reported to have a higher level of psychological distress than the general population matched by age and year of training and exam period. Similarly, according to qualitative studies described elsewhere (Morneau-Sévigny *et al.*, 2013; Kadri *et al.*, 2011; Radcliffe and Lester, 2003), the 12 transition periods, the lack of support from supervisors, the practical application of theoretical knowledge and the pressure associated with work are stressors for medical students. According to a study of 7,800 Canadian students, 30% of academics report experiencing psychological distress (Adlaf *et al.*, 2001). In comparison, 20.74% of the general population aged 20

Table 1: Levels of anxiety and depression (HADS) according to students not taking exams and students during exams

		Students without exams	Students during exams
Anxiety levels	No anxiety	10	1
	Insignificant anxiety state	5	2
	Significant anxiety state	0	12
Degree of depression	No depressive state	13	1
	Beginning of depressive state	2	7
	Highly depressed state	0	7

Table 2: Variation in the number of total leukocytes (cell $\times$ 10^3) and of leukocyte subpopulations in (%) among students without exams compared to students during exams

Leucocytes	Before exams	During exams	Meaning
WBC	7668 $\pm$ 1884	5896$\pm$2375	*(p : 0.03)
Neutrophils	51,513 $\pm$ 5,159	60,513 $\pm$ 8,510	** (p : 0.002)
Basophils	0,5067 $\pm$ 0,2520	0,6000 $\pm$ 0,3665	Non significant
Monocytes	7,220 $\pm$ 4,073	6,960 $\pm$ 0,659	Non significant
Eosinophils	2,107 $\pm$ 0,980	2,247 $\pm$ 1,939	Non significant
Lymphocytes	40,653 $\pm$ 4,662	33,927 $\pm$ 8,676	** (P : 0.002)

or more reports experiencing distress (Caron and Liu, 2010; Ridner, 2004). Scientific research on stress has more recently addressed the role of the brain in the response to stress. Different areas of the brain are involved in organizing responses to aversive or threatening stimuli, and these areas interact widely with one another. Hypothalamic neurons, for example, are sensitive to internal physico-chemical stimuli, external physical stimuli and psycho-social stimuli. The stress response is immediate to a large extent due to the release factor of corticotrophin (CRF), secreted mainly by the paraventricular nucleus of the hypothalamus (Koolhaas *et al.*, 2011). In our study we noticed that stress during exams caused a change in the distribution of different types of white blood cells, or we found a decrease in total white blood cells and monocytes and lymphopenia with an increase in the rate of neutrophils and other polymorphonuclear drugs in students during exams compared to controls outside exams. Numerous studies carried out on laboratory animals exposed to various types of stress show that the immune responses are very sensitive to stressors.

For example, exposure to unavoidable or unpredictable electrical shock decreases the proliferation capacity of T cells in rats, while electrical shock has no effect when it is controllable or predictable. The same phenomena are observed in human subjects exposed to various life events (bereavement, illness of a parent, marital difficulties, competition) (Koolhaas *et al.*, 2011). Rodent studies have shown that stress-induced changes in the number of blood leukocytes are characterized by a significant decrease in the number and percentages of lymphocytes and monocytes and by an increase in the number and percentages of neutrophils (Dhabhar *et al.*, 2012). Flow cytometric analyzes revealed that the absolute number of peripheral blood T cells, B cells, natural killer cells (NK) and monocytes showed a rapid and significant decrease (40 to 70% below the baseline) during stress (Dhabhar *et al.*, 2012). In addition, stress-induced changes in the number of leukocytes have been shown to be quickly reversed when stress is stopped (Dhabhar *et al.*, 2012). Unlike animal studies, human studies haveshown that stress can increase rather than decrease the number of blood leukocytes (Bosch *et al.*, 2003) . This apparent contradiction can be resolved by taking into account the following factors: First, stress-induced increases in the number of blood leukocytes are observed as a result of stress conditions that primarily result in activation of the sympathetic nervous system. These stressors are often short-lived (a few minutes) or relatively mild (eg, speaking in public) (Goldstein and McEwen, 2002). Second, the increase in the total number of leukocytes can be explained by increases induced by stress or catecholamines in granulocytes and NK cells (Estruel-Amades *et al.*, 2020). Third, the increase in glucocorticoid hormones caused by stress or pharmacology induces a significant decrease in the number of lymphocytes and monocytes in the blood (Reiske *et al.*, 2019) . Thus, the stress conditions which cause significant and sustained activation of the hypothalamic-pituitary-adrenal (HPA) axis lead to a decrease in the number of blood leukocytes. The results of a study by a research team in the United States are consistent with the impact of the stress factor and the variance it induces on the numbering of leukocytes in our group of students. The latter assert that the parabolic flight maneuvers lead to changes in the number of different leukocyte subpopulations.

The naive and memory T / B lymphocyte subpopulations were reduced under the effect of gravitational stress, a decrease in the number of basophils and eosinophils was also observed. Only circulating neutrophils that have been increased during parabolic flight (Stervbo *et al.*, 2020). Exposure to acute noise stress leads, according to a previous study, to a very significant decrease in the total number of leukocytes compared to control and a significant improvement in the number of neutrophils positive for phagocytosis of Candida in the group exposed to acute noise stress compared to control. This study revealed that exposure to stress due to acute noise increases markers of stress and produces changes in various parameters of the functioning of the immune system (Sridevi *et al.*, 2016). Cross-sectional observational studies have repeatedly linked inflammatory status to depression, which has been associated with greater severity of specific neuro-vegetative symptoms, such as sleep and appetite. Various studies have highlighted the importance of immune cells in immune monitoring at the CNS level, low levels of immune cells contribute to neurodegeneration. They have been found to be associated with anxiety depression in people with depression, increased severity of mood symptoms, and increased mood symptoms specific to bipolar disorder (Köhler-Forsberg *et al.*, 2017). The production of epinephrine and the administration of steroids can cause neutrophilia as is the case with emotional and physical stress; which dramatically increases the level of steroid adrenocorticoid hormones. Neutrophilia usually occurs within minutes of physical exercise or the source of some stress. This results in pairing from the release of the pool of marginal neutrophils into the blood stream (Ok *et al.*, 2018).

In view of the above discussion, it has been proposed that acute stress induces an initial increase followed by a decrease in the number of blood leukocytes. Stress conditions that cause activation of the sympathetic nervous system, especially conditions that induce high levels of norepinephrine, can cause an increase in the number of circulating leukocytes. These conditions can occur at the start of a stress response, very short-term stress (in minutes), mild psychological stress, or exercise. On the other hand, the stress conditions which cause the activation of the HPA axis induce a reduction in the number of circulating leukocytes. These conditions often occur during the later stages of a stress response, long-term acute stressors (order of hours) or severe psychological, physical or physiological stress (Silverman *et al.*, 2005). An elegant and interesting example to support this hypothesis comes from Schedlowski and his colleagues, who measured changes in the number of blood T and NK cells as well as the plasma levels of catecholamines and cortisol in paratroopers (Schedlowski *et al.*, 1993). The measurements were taken 2 hours before, immediately after and 1 hour after the jump. The results showed a significant increase in the number of T cells and NK cells immediately (minutes) after the jump which was followed by a significant decrease 1 hour after the jump. An early increase in plasma catecholamines preceded an early increase in the number of lymphocytes, while a more delayed increase in plasma cortisol preceded the late decrease in the number of lymphocytes (Rajda *et al.*, 2002) .

Importantly, changes in NK cell activity and antibody-dependent cell-mediated cytotoxicity were closely parallel to changes in the number of blood NK cells, thus suggesting that changes in leukocyte numbers could be an important mediator of the changes apparent in the "activity" of leukocytes. Likewise, Rinner and his colleagues (1992) have shown that a short stress factor (manipulation of one minute) induces an increase in the proliferation induced by the mutagens of T and B cells obtained from peripheral blood, while a factor longer stress (immobilization of 2 hours) induced a decrease in the same proliferative responses (Rinner, 1992). In another example, Manuck and his colleagues showed that acute psychological stress induced a significant increase in the number of cytotoxic T lymphocytes in blood only in subjects who presented a catecholamine and increased cardiovascular reactions to stress (Segerstrom and Miller, 2004) When interpreting data showing stress-induced changes in functional tests such as lymphocyte proliferation or NK activity, it may be important to keep in mind the effects of stress on the leukocyte composition of the compartment in which an immune parameter is measured (Dhabhar and

Viswanathan, 2005). This stress-induced decrease in the number of blood and spleen leukocytes may contribute to the stress-induced acute suppression of immune function in these compartments.

CONCLUSIONS

This study has investigated the effect of stress and anxiety during student's exams. The results have shown a relationship between the degree of anxiety and the emergence of depressive disorders, and the threat of failure in the exams at the end of the year. It is also found that stress during exams caused a change in the distribution of different types of white blood cells (a total decrease in white blood cell counts with neutropenia and lymphopenia, and an increase in monocyte and other types of polymorphonuclear levels.

The study concludes that the modification of the blood formula and the alteration of the proliferative responses of the leukocytes constitute evidence of the action of stress on the immune system.

ACKNOWLEDGEMENT

Authors are thankful to all participants in this study.

Conf lict of Interest

Authors declare no conflict of interest.

Funding Source

Authors have not received any financial supports.

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