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SHORT COMMUNICATION

The Effect of Exam Stress on Serum IL-6, Cortisol, CRP and IgE Levels

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Abstract

Background: Stress has determining effects on the immune response. This study was undertaken to determine the effect of exam stress on serum IL-6, cortisol, CRP and IgE levels of medical students.

Methods: Thirty five university medical students in Southern Iran were enrolled. Two blood samples were provided from each participant one month (first stage) and one hour (second stage) before the exam at 1.00 PM. The students completed the SpielBerger State-Trait Anxiety Inventory (STAI) questionnaire. IL-6, cortisol, CRP and IgE changes were determined in these students.

Results: The mean age of the students was 21 years. The exam stress resulted in a significant decrease in the IgE level and a significant increase in the cortisol level.

Conclusion: The exam stress can result in a decrease in the IgE level and a significant increase in the cortisol level. So, these changes may indicate the alterations of immunological status and presence of stress in an immunosuppressed individual, affecting his/her health.

Keywords: Stress; Immune system; IL-6; IgE, CRP; Cortisol

Introduction

Stress is an extremely adaptive phenomenon in human, contributing to his/her survival, activities, and performance.[1] Physical and psychological stresses can induce a wide range of immunological alteration in the cell mediated and humoral immunity.[2] Stressors may influence the immune function via their impact on neuroendocrine, autonomic and central nervous system.[3] Psychological stresses may influence the immunological functions both indirectly through hormonal changes, and directly through nervous regulation during brief but acute stressful periods.[4] Exposure to psychological stressors can modulate the primary antibody response.[5,6] In humans, a range of stressful events have been associated with lowering the immune system functioning, including examinations, battle task, vigilance, sleep deprivation and divorce. Any psychological or behavioral event such as anger, anxiety, and especially depression that is capable of affecting the autonomic nervous system's activity or hormonal activity is also able to influence the immune system, showing that the brain and the immune system are sufficiently well connected and influenced by each other.[7] Academic examination stresses were reported to have a significant impact on the student's well-being,[8] and are associated with changes in the mental and physical health such as increasing anxiety, increasing negative mood and changes in the immune functioning.[9] This study was performed to determine the effect of exam stresses on serum IL-6, cortisol, CRP and IgE levels of medical students in Southern Iran.

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Materials and Methods

Thirty five medical students of Fasa University of Medical Sciences in Southern Iran entered this study based on the statistical formula,[10]

$$n = \left[\frac{z_{\frac{\alpha}{2}}\sigma}{d} \right]^2$$

Where α=0.05, d=0.3 and SD=0.9 .There were 19 males and 16 females in the same level of education (5th semester) and all participated from 2.00 to 4.00 PM in an identical exam (final exam of the immunology course). All the students were informed about the study and gave a written consent to participate in the study. None of the students had major depression, consumed immune suppression medication or any drug affecting the immune system, suffered from any immune suppression diseases, and had any atopic allergy according to the IgE level. A control group was selected in which subjects with no history of allergic diseases were matched to the case group for age and sex. To assess the extent and source of any possible stresses, the participants completed the Spielberger State-Trait Anxiety Inventory (STAI) questionnaire in two stages, one hour and one month before the exam. STAI consisted of 20 items reflecting the present person's feelings and factors that might influence the anxiety level. The score range was from 20 to 80 and the higher the score, the greater the level of anxiety.[11]A Persian version of the STAI was provided with the permission of the authors researching panic attacks in Iranian university students; it was a review of an initial translation,[12] and had a high internal consistency (α=.91) and an acceptable convergent validity with the Persian version of STAI scale.[13]

Two blood samples were provided from each participant, one month (first stage) and one hour (second stage) before the exam at 1.00 PM. Cytokine levels in the blood were determined by ELISA, using monoclonal antibodies specific for IL-6 (Bender med systems GmbH). The sensitivity of the ELISA was 4.3 pg/ml. The serum cortisol levels were determined using a commercial ELISA kit (IBL-Hamburg GmbH). The cortisol responded in the kit buffer and was analyzed by quantitative competition ELISA. IgE was determined using the IgE Kit (Genesis GD09, UK). The concentration of IgE was measured using ELISA plate reader (Emax, Molecular Devices) and the concentrations were calculated based upon standard curves using the SOFTmax for Windows software package (Molecular Devices). C-reactive protein (CRP) was evaluated by serum agglutination test. The study was approved in the Ethics Committee of Fasa University of Medical Sciences and a written consent was given by each participant.

SPSS software (Version 11.5, Chicago, IL, USA) was used for data analysis. Paired *t* test was used to compare the results. The level of statistical significance was set at $p<0.05$.

Results

The mean age of the students was 21±1.2 years (16 females and 19 males). There was no significant difference between genders in terms of response to stress. The first stage serum cortisol level was significantly lower than the second stage ($p=0.020$) but for the serum level of IgE, the second stage level was significantly lower ($p=0.031$). C-reactive protein (CRP) level was positive in 20% of the students but the difference was not significant at the first and second stages ($p=0.466$). The stress mean score based on STAI was significantly more in the second stage ($p=0.001$) (Table1).

Table 1: The correlation between immunological factors, cortisol, anxiety inventory and the exam stress

Immunological factors test	IL-6(pg/ml)	IgE (ng/ml)	IgE (ng/ml) (Control)	Cortisol (nmol/l)	CRP (positive)	Anxiety (STAI)
One month before exam (first stage)	40.32±7.1	68.33±11	62.6±9.6	78.6±6.9	0/30	36.33±1.7
One hour before exam (second stage)	36.06±5.7	50.51±8.1	61.2±9.1	91.82±7.5	6/30	45.43±1.9
p value	0.680	0.031	0.241	0.020	0.466	0.001

STAI=State-Trait Anxiety Inventory, CRP=C-reactive protein

Discussion

Under stressful conditions, the hypothalamus releases corticotrophins into the blood circulation and when it reaches the anterior lobe of the pituitary gland, adrenocorticotrophic hormones are released into the blood circulation. When this reaches the adrenal gland, glucocorticoids are released which are chemicals that modulate or regulate the immune response. Since lymphocytes have receptors for glucocorticoids as well as other steroid hormones associated with responses to stress, the brain and immune system are sufficiently well connected to one to influence the other.[14]

The examination is also one of the stressful events associated with lowered immune system function.[14] Academic examinations have often been used in stress research because they are predictable, standardized, and discrete examples of real-life stressors. It was demonstrated that this stress induced a significant neurohormonal change.[15,16] Psychological assessments indicated that although none of the students exhibited clinical levels of anxiety or depression, there was a small but significant increase in their emotional distress, as indicated by an increase in STAI (30±0.9=low-stress versus 33±0.8=high-stress) during the final examination.[10] A significant correlation was also found between psychological measures and immunological parameters. In our study, the stress mean score at the first stage based on STAI was significantly more than the second stage (p=0.001). In another study, the mean STAI score was reported 42.66 without any significant gender differences12 which is similar to our results.

To a student, the prospect of sitting for an examination could be identified as a stressor and the resulting emotional and physiological state could be described as an exam stress.[8] Our results showed that one hour before the exam, the cortisol level had a significant increase (p<0.05). It was shown that individual differences in cytokine responses were associated with sympathetic reactivity[17] and inflammatory cytokines responded to acute mental stresses in humans with a delayed increase which explained our results.[18] The difference may be due to the time of data collection as in our study; blood samples were provided one hour prior to the exam, whereas in other study it was in the midsemester.[18] Glucocorticoids can down-regulate the immune activity, but acute stresses were reported to increase the cortisol level,[19] which is compatible with our findings as well.

Several immune competent cells bear adrenergic receptors, although the distribution of specific types of these receptors are not uniform and they create the opportunity for fine-tuned regulation of the immune response; that is, Th1 and B cells express β2-adernergic receptors. Their activation is typically associated with increased intercellular cAMP and inhibition of cell function.[20]

Our results showed that one hour before the exam, the IgE level significantly decreased (p<0.05) which may be due to the changes in the immune system in stressful situations and a decrease in humoral immunity. However, with respect to the time required for IgE synthesis, one of the reasons for the decrease in IgE secretion could be stress inducing conditions few days before the examination. In contrast, in relation to the IgE level, no difference was observed in the control group (p=0.241) (Table1).

Regarding the effects of stress inducing agents on the innate immunity and acquired immunity mechanisms,[21] they could also affect inflammatory cytokines, but such an effect would be associated with a delay in inflammatory cytokine responses. Glaser *et al.* showed that T-cell killing by memory T lymphocytes of EBV transformed autologous B lymphocytes and also declined during examination periods. The increase in the incidence of self-reported symptoms of infectious illness was also associated with examination periods.[22] Szczeklik and Jawien showed that IgE might be involved in acute phase response to tissue injury.[23] Rosalind *et al.* showed that stress was also associated with a high total IgE level[24] which was different from our results, because the patients were already exposed to specific allergens as well as stresses, resulting in an increase in IgE level.

Kang et al. showed that during examinations, there was a significant decrease in IL-2 and IFN-Ɣ and a significant increase in IL-6 levels.[25] Their results are different from ours as we observed a slight decrease in IL-6 level (p=0.679). The difference may be due to a down-regulation of Th1 and a selective up-regulation of Th2 cytokines during a stressful exposure observed in Kang et al.'s study.[25] whereas others reported no changes in IL-6 and IL-2 levels following exposure to acute stressors.[26,27]

Therefore, it could be inferred that inflammatory cytokine response to the released factors of the nervous system requires a longer time. On the other hand, the level of IL-6 increased in those who experienced severe stresses such as major depression, heavy exercise, increased weight and sleep disturbances.[28,29] Examination can probably make less stress than the

above-mentioned factors, and needs more time to elevate the level of IL-6.

Parasympathetic withdrawal during naturalistic stress may shift the balance between anti-inflammatory and pro-inflammatory cytokines, and in patients with elevated concentrations of CRP who are known to have a greater degree of atherosclerotic burden and increased plaque inflammation, it provokes plague rapture.[30] Changes in the innate immune system, as measured by acute phase proteins of hepatic origin, seem to be a feature of posttraumatic stress disorder (PTSD). Furthermore, the inverse correlation between RHEA-s, which has been shown to be changed in PTSD and the acute phase proteins, is of interest.[31] There was a 20% change in the CRP level of the students but the difference was not significant in both stages.

High levels of serum basal cortisol, and changes in the level of IgE antibody, plasma concentration of IL-6 and a shift in the balance of Th1 and Th2 can affect the immune responses. So, during stress, a decrease in humeral immunity may occur.[32] Höglund *et al.* showed that other stress-induced immune changes were unique to atopic individuals, such as a skewed Th1/Th2 ratio and reduced NK cell numbers, indicating that some pathogenic mechanisms in atopics may be more strongly affected by stress than other factors.[33]

Our results show that the exam stress can result in a decrease in IgE level and a significant increase in the cortisol level. So, these changes may indicate alterations of immunological status and presence of stress in an immune suppressed individual affecting his/her health.

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