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Academic stress-induced changes in Th1- and Th2-cytokine response

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ABSTRACT

Psychological stress stimulates physiological responses releasing catecholamines and corticoids, which act via corresponding receptors on immune cells, producing a shift in the cytokine balance. These responses are variable depending on the nature of stressors. The effect of the academic stress on the production of the Th1-cytokines (TNF-α, IFN-γ, IL-1β, IL-2, IL-6 and IL-8) and Th2-cytokines (IL-1ra, IL-4, IL-5 and IL-10) on 35 medical/health sciences students after completing their questionnaires was investigated. Blood samples were taken at three stages; baseline stage at the beginning, midterm and final academic examination stages. Plasma cortisol and cytokines were measured during the three stages. The last two stages were compared with the baseline non-stress period. Results of the stress induced during the final examination stage were the highest with a significant increase in cortisol release, IL-4, IL-5 and IL-1ra release with a shift in Th1:Th2 cytokines balance towards Th2. Whereby, the midterm stage did not show significant reduction in Th1-cytokines except for TNF-α, with an increase in IFN-γ level that was reduced in the third stage. Th2 cytokine, IL-1ra, had positive correlations with Th1 cytokines; IL-2 and IFN-γ in the second stage and IL-6 cytokine in the third stage. Cortisol was positively correlated with IL-8 in the last stage and heart rates had negative correlation with IL-10 in the first and last stages. Findings of this study indicate that exam stress down-regulates Th1 with a selective up-regulation of Th2-cytokines. In conclusion, Cortisol might have a role in suppressing the release of Th1- mediated cellular immune response which could increase the vulnerability among the students to infectious diseases.

1. Introduction

The relationship between psychological stressors and immunity has gained lots of interest among scientists and clinicians in the field of health psychology and psychoneuroimmunology. Studies on psychological stress found that depending on the type of stress, stress hormones may suppress or enhance the immune function. Some studies suggested that stress hormones such as glucocorticoids and catecholamines inhibit the production of proinflammatory cytokines. Whereby, they stimulate the production of the anti-inflammatory cytokines (Chrousos, 2000; Connor et al., 2005; Dhabhar et al., 2014; Elenkove and Chrousos, 2002). Other studies indicated that stress hormones in stress are potential mediators of exhaustive exercise-induced immunosuppression (Kohut et al., 2005). On the other hand, researchers also suggested that certain local responses and under certain conditions, stress hormones actually may boost regional immune responses by increasing the production rate of the pro-inflammatory cytokines such as Interleukin-6 (IL-6), Tumor necrosis factor - alpha (TNF-α) and Interferon (IFN)-gamma (Maes et al., 1998; Elenkove and Chrousos, 2002). Moreover, recent studies indicated that psychological stress causes sympathetic responses in the autonomic nervous system leading to several effects such as reducing heart rate (Föhr et al., 2016, Lee et al., 2014).

Cytokines are small proteins released by white blood cells and modulate the balance between humoral and cell-based immune responses. They have a specific effect on the interactions and communications between cells. CD4-expressing T helper cells are the major source for cytokine production and regulation and they are of two subtypes of cells known as helper T cells type 1 (Th1) and type 2 (Th2) (Chen, 2007). Th1 can evoke cell-mediated immunity and phagocyte-dependent inflammation and make disease worse by producing proinflammatory cytokines such as IFN-γ, IL-2, TNF-α, IL-6, IL-8 and IL-1β. Th2 cells are known to evoke strong humoral-mediated immunity with antibody production and eosinophil accumulation by producing anti-inflammatory cytokines

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such as IL-1ra, IL-4, IL-5 and IL-10 which counteract the Th1 mediated response (Chen, 2007, Shinkai et al., 2002). Excessive proinflammatory responses as in acute inflammation can lead to uncontrolled tissue damage, fever, inflammation and, in some cases, shock and death. Whereas, excessive Th2 response, as in chronic inflammation, is associated with allergies and atopy (asthma, eczema, allergic rhinitis & allergic conjunctivitis). The balance between Th1 and Th2 responses is needed for optimal health whereas any disturbance would influence many pathological processes (Chen, 2007; Dhabhar et al., 2014; Kidd, 2003; Kim and Maes, 2003). Therefore, it is very important to distinguish between the types of psychological stressors regarding their duration, frequency and severity to understand their impact on the Th1 and Th2 cytokines.

Several models have been studied in this field focusing on the immunosuppressive consequences of stress on the immune system (Dhabhar et al., 2014; Segerstrom and Miller, 2004). One of the studied models was on the academic stress such as on the effect of studying or of examination periods on immunity and cytokine release (Kim and Maes, 2003). Results were variable, some indicated that examination stress down-regulates immune functions such as the lymphocyte proliferation, the production of IFN-γ, the activity of natural killer (NK) cells, the mucosal wound healing and the salivary immunoglobulin A (IgA) (Glaser et al., 1986; Marucha et al., 1998; Murphy et al., 2010; Rojas et al., 2002). Other studies showed immune activation in response to examination stress such as enhancing salivary IgA in students during acute stress of an imminent examination (Murphy et al., 2010). Also, the levels of phytohemagglutinin (PHA)-stimulated IL-2 production and lymphocyte proliferative response to PHA, were higher during an examination period compared to a non-examination period (Koh et al., 2001; Koh et al., 2006). In 1998, Marshall indicated that stress induced by exams, among healthy medical students, changed the immune response by shifting the balance of Th1 and Th2 cytokines towards Th2 cytokine response.

Several types of academic stressors, such as too many assignments, academic commitments, financial pressure, students' accommodation, taking and studying for exams, study loads, competition attitudes and other relative stressors, have been identified by researchers (Assaf, 2013; Fairbrother and Warn, 2003). The degree of stress experienced by students may differ from one university to another depending on the university system, environment and culture in the country. But, almost all, consider academic examination as a naturally occurring psychological stressor. In 2014, Dhabhar evaluated the range of effects of stress on immune function, and discussed how these effects may promote immunoprotection versus immunopathology. In our previous survey study done at The University of Jordan, the negative relations between academic stress and health outcomes on Pharmacy College students were determined (Assaf, 2013). In this study, we wanted to analyze the influence of the academic stress experienced by medical/health sciences students at The University of Jordan on their immune system, and to determine the direction of the immune balance whether it would enhance or suppress their immune response at the beginning, mid of the semester and in the final exam periods. So, the aim of this study is to determine the psychological academic stressors and measure the levels of the stress hormone (Cortisol) and the pro-inflammatory (Th1) and anti-inflammatory (Th2) cytokines released in the blood of the medical/health sciences students at The University of Jordan. The secretion of cortisol hormone was measured during the three stages as a marker of psychological stress. Then, to compare changes in the plasma levels of the stress hormone (cortisol) and cytokines (Th1 and Th2) released during the mid of the semester and in the final exam period with the baseline non-stress period at the beginning of the fall semester.

2. Materials and methods

2.1. Participants

The ethical approval for the study was obtained from the Institutional Review Board of Jordan University Hospital. The study was performed over the course of an academic semester between September 2010 and February 2011. Volunteers were recruited from a group of medical/health sciences students at The University of Jordan (Amman, Jordan), excluding asthma suffering students and the first-year students who have not yet adjusted to the University environment. A total of 40 students responded positively and met the inclusion criteria, of whom 35 complied fully with the requirements of this study in the three stages (34 students from school of Pharmacy and one student from school of Medicine) comprising 88.6% females and 11.4% males. The purpose and procedures of the study were explained to all potential subjects and an informed consent was obtained from those who decided to participate. The clinical parameters of resting blood pressure and heart rate for each participant were taken at the beginning and at the end of the study. They were measured twice each over a 5 min interval and the average of the readings was recorded.

The study was done along three stages, the first week of the semester, the mid of the semester and during the academic final examinations of the fall semester of 2010/2011. Volunteers were all unmarried, non-smokers, with no chronic disease and with no recent acute illness or use of antibiotic, steroidal, or anti-inflammatory medications. To protect their anonymity, designated numbers for the participants were placed on the questionnaire forms and on their blood samples labels.

2.2. Procedure

This study was carried out using a longitudinal design. Blood samples collection was carried out through the three stages during the period September 2010 - January 2011. The results of the last two stages were compared with the baseline first stage. An interviewer administered a questionnaire to the participants. The questionnaire was designed to reveal information about stress as perceived by participants at the beginning and end of the academic fall semester of 2010/2011. After providing a written informed consent, the respondent students completed a pre-validated short questionnaire survey. The questionnaire was developed by reviewing available surveys in the literature which used to measure students' academic stress as indicated in the previous study (Assaf, 2013). Validity and reliability of the questionnaire were evaluated by an expert committee of one clinical immunologist, one clinical pharmacist and one statistician. This was to ensure its applicability relevant to the medical/health sciences students at The University of Jordan. Internal Consistency Reliability was tested by the Cronbach's alpha Coefficient and they all were above 0.65.

The questionnaire was handed to the responding medical/health sciences students, after briefing, discussing and answering questions regarding this survey by the researcher and the research assistants. It was administered twice: once at the beginning of the semester and again in the academic final examinations period. The purpose was to indicate the psychological status of the respondents at the beginning of the semester and during the semester. Blood samples were collected within the three periods.

2.3. Psychological assessment

To detect the effect of academic stress on the students' immune system taking into consideration that they may not be under the

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