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Association of physical activity and IL-10 levels 20 years after sulfur mustard exposure: Sardasht-Iran cohort study

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ABSTRACT

IL-10 is an anti-inflammatory cytokine that is important in the regulation of inflammatory processes in different conditions. Sulfur mustard (SM) intoxicated patients are suffering from different inflammatory diseases in their lung, skin and eyes. Physical activity (PA) is reported to control inflammation by reducing pro-inflammatory and inducing anti-inflammatory cytokines. Our previous study revealed lower PA and more sedentary lifestyle among SM exposed population. This study aimed to determine the relationship of PA with IL-10 production in SM exposed subjects. Baseline, mitogen-induced and the serum levels of IL-10 were evaluated.

In a historical cohort study, Sardasht-Iran Cohort Study (SICS), 372 SM exposed participants were studied 20 years after exposure and were compared with 128 unexposed control participants. The Global Physical Activity Questionnaire (GPAQ; developed by WHO) was used to obtain a self-reported measure of physical activity. Whole blood culture supernatants and serum samples were used for IL-10 measurement by ELISA technique.

In both the control and exposed groups mitogen-induced IL-10 production was significantly elevated with severity of PA intensity ($p<0.05$). In the control subjects with moderate PA intensity, the mitogen-induced IL-10 production was higher than the corresponding in the exposed group ($p<0.05$). In the exposed group, mitogen-induced IL-10 production had significant positive correlation with total PA, total transport PA, total recreational PA and total moderate intensity work ($p<0.05$). The positive relationship between high PA and the levels of anti-inflammatory cytokine IL-10 indicates a need to encourage a more active lifestyle among the SM exposed subjects who have various inflammatory complications.

1. Introduction

Sulfur mustard (SM) affects many organs including gastrointestinal and respiratory tracts, skin, eyes, hematological, endocrine, neuromuscular and immune systems [1]. The extent of tissue injury in these organs depends on severity and duration of the exposure [2]. The civilians of Sardasht were exposed to SM in 1987 [3]. SM exposed individuals suffer from various disabilities and inflammatory diseases such as cutaneous, respiratory and ocular inflammations [4–6]. For people with disabilities promoting healthy lifestyle behaviors such as physical activity (PA) leads to prevention of secondary conditions (e.g. muscle atrophy and obesity) [7,8].

Recently we reported a significant reduction of total PA and high prevalence of sedentary lifestyle in SM exposed population in Sardasht-Iran Cohort Study (SICS) [9,10]. It has been shown that regular PA can improve immune function and modulate inflammatory responses [11,12]. Cytokines are a group of proteins that mediate inflammatory

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responses to infections and tissue damages [13]. Cytokines are divided into two major groups, pro- and anti-inflammatory cytokines. IL-10 is one of the main anti-inflammatory cytokines [14] that is produced by various types of activated immune cells such as T-helper 2 (Th2) cells, B cells, macrophages, monocytes and keratinocytes [15]. It seems that its major biological function is to restrict and terminate inflammatory responses [16]. Physiological stimuli including heavy exercise and stress hormones can modify production of cytokines [13].

Sedentary lifestyle and physical inactivity are associated with an increase of inflammatory markers [17]. Also it has been suggested by several data that an increase of PA and a decrease of energy intake could effectively diminish overall inflammation [18]. Consistent with the anti-inflammatory role of PA it has been shown that plasma concentrations of anti-inflammatory cytokines like IL-1ra, IL-4, IL-10, IL-12p40 and MCP-1 increase after different types of exercise [19].

Furthermore it has been demonstrated that greater amount of regular PA is associated with reduction of pro-inflammatory IL-6 levels and elevation of IL-10 levels in healthy older males [20]. Aerobic exercise training without significant weight loss has been reported to exert anti-inflammatory effects in type 2 diabetes mellitus patients due to IL-10 increment and down regulation of IL-18/IL-10 ratio [21]. Cyclic exercise over months causes a significant increase in plasma anti-inflammatory signal molecules including interleukin-10 and adrenocorticotropin. The aim of the present study was to evaluate the correlation of PA and the levels of anti-inflammatory cytokine IL-10 in SM exposed population and its comparison with the control group.

2. Materials and methods

2.1. Study design and participants

This study is a part of the SICS, a comprehensive historical cohort study of Sardasht SM exposed population designed to study the long-term clinical complications of SM exposure and their underlying mechanisms. The details of the methodology of SICS have been previously described [22]. The two study groups consisted of the SM exposed ($N=372$) and the unexposed control groups ($N=128$). The age range covered by the study was 20 to 60 years of age. This study was approved by the Ministry of Health of Iran, Shahed University and the Board of Research Ethics of Janbazan Medical and Engineering Research Center. A written informed consent was obtained from all the subjects in the study.

2.2. Physical activity assessment method

The Global Physical Activity Questionnaire (GPAQ; developed by WHO) was used to obtain a self-reported measure of the physical activity. The questionnaire has been validated in nine countries including the Islamic Republic of Iran [23]. Previous studies have demonstrated that the GPAQ is a valid and reliable tool for this purpose [24]. Translation and back translation procedure based on Brislin's model [25] was used to develop the culturally equivalent questionnaire. In addition two bilingual experienced health educators translated the questionnaire into Persian, and another two bilingual health educators back translated it independently. The researchers and the four translators discussed the clarity of the translation work and examined discrepancies between the two versions, and finally amended a few items to ensure the appropriateness of the translation [26]. This instrument collects information about participating in PA in three domains and sedentary behavior. The domains are activity at work; travel to and from places and recreational activities [27].

The participants were asked to recall the number of times they usually take part in at least 10 min of mild, moderate and vigorous PA in an average week by the health education experts. Examples were provided for intensity of the physical activity. The raw frequency scores were then multiplied by a corresponding metabolic equivalent (MET) value (4 for moderate MET value, 8 for vigorous MET value and 4 for cycling and walking MET value) to provide a total PA score. For the purposes of this study, the participants were asked to consider a typical week.

2.3. Serum preparation

Peripheral blood was drawn into Vacutainer tubes (BD Biosciences). The sera were separated by 20 min centrifugation at $2000\times g$ (4 °C), aliquoted, labeled and were kept frozen at −80 °C until laboratory measurements.

2.4. Whole blood culture

Peripheral blood samples were drawn into Quantiferon Mitogen and Nill tubes (Cellestis Gm bH, Darmstadt, Germany) for measurement of mitogen-induced, and baseline IL-10 cytokine production in the supernatant of whole blood cell culture respectively. Mitogen was phythohemaglutinin (PHA). Quantiferon tubes make cytokine evaluation possible to be carried out in the field as the whole blood sample is drawn into the tubes, the cells are allowed to proliferate and secrete cytokines through incubation in 37 °C for 24 h without the need of CO_2. The tubes were then centrifuged and supernatant were harvested and divided into aliquots and kept frozen in −80 °C till used. Since 1 ml of the whole blood sample was used for the cytokine production, mitogen-induced cytokine production for each person was normalized based on CD3 absolute number, and baseline cytokine production for each person was normalized based on WBC count [22]. Furthermore baseline values were subtracted from mitogen values for each sample.

2.5. Cytokine measurement

Human IL-10 DuoSet® ELISA Development kit (R&D Systems) was used to measure IL-10 levels in the sera and cell culture supernatants. Primary antibody was mouse anti-human and biotinylated goat anti-human was secondary antibody. Standards of the kits were diluted with 1% BSA in PBS. Wash buffer was 0.05% Tween 20 in PBS, and 1% BSA in PBS used as block buffer. Used PBS in wash and block buffer contained 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, and 1.5 mM KH_2PO_4. ELISA reader and washer were Stat-Fax 2100 and Stat-Fax 2600 (USA) respectively.

2.6. Statistical analysis

Data was presented as median (first–third quartiles). Comparison of IL-10 levels among groups was performed using the Mann–Whitney test. Correlation between IL-10 in different ages with PA was computed with Spearman rank correlation coefficient. The multiple stepwise regression test was used to find which PA domains were correlated with IL-10 (separately in mitogen-induced, baseline and serum). Adjusting analysis for effect of age (years) and exposure (control = 0, exposed = 1) this factor was inserted as a first block in a two level stepwise regression analysis. All of PA parameters were inserted as second block in this analysis. Un-standardized regression coefficient and its standard error of models were reported.

p-value less than 0.05 was considered as statistically significant. Analyses were performed with the SPSS 13 (SPSS Inc. Chicago, Illinois, USA).

3. Results

3.1. Physical activity and IL-10 levels in the control and SM exposed groups

As shown in Table 1 baseline IL-10 production was not significantly different between various PA intensity levels as well as PA domains in both study groups. Exposed subjects with low and