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Alterations in serum levels of inflammatory cytokines (TNF, IL-1alpha, IL-1beta and IL-1Ra) 20 years after sulfur mustard exposure: Sardasht-Iran cohort study

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ABSTRACT

Mustard gas, even in low doses, has the ability to inflict damage in multiple organs especially the skin, eyes, as well as the respiratory tract. This damage may cause many complications which persist during the lifespan of exposed subjects. Pro-inflammatory cytokines including TNF, IL-1α, IL-1β and IL-1Ra cause systemic inflammatory reactions and numerous changes including altered cell signaling and migration, changes in cytokine production and fever. The aim of this study was to determine the serum levels of these cytokines in subjects who were exposed to mustard gas 20 years ago in comparison with an unexposed control group. In this historical cohort study 368 sulfur mustard (SM) exposed participants from Sardasht and 126 age-matched unexposed volunteers from Rabat (a nearby town) as controls were chosen by a random systematic sampling. The serum concentrations of IL-1α, IL-1β, IL-1Ra and TNF were measured by a sandwich ELISA technique. Median of the serum levels of cytokines TNF, IL-1α, IL-1β and IL-1Ra in the control group was 23.79, 1.89, 1.91 and 32.9 pg/ml respectively, while in the SM-exposed participants these values were 11.11, 0.81, 1.73 and 26.7 pg/ml respectively. The serum pro-inflammatory cytokine levels were significantly lower in the exposed group than in controls ($p<0.01$). There was also significant positive correlation between concentration of all of mentioned cytokines, the strongest being between IL-1β and TNF ($r=0.809$ in the control group). The observed down-regulation of pro-inflammatory cytokines should be considered in interpretation of diagnosis and therapeutic measures taken to improve clinical complications.

1. Introduction

Sulfur mustard (SM) is an alkylating agent, which has been reported to induce short and long term adverse effects in multiple organs especially the skin, eyes and respiratory tract. Basic and molecular mechanisms involved in SM clinical manifestations are unclear and clinical management strategies especially the treatment and diagnostic protocols are not well defined in the cases of mustard gas-injured patients [1,2]. Basic studies regarding immunological status of long term effect of mustard gas exposure are rare and major questions in this regard remain unanswered [3,4]. Although some recent studies indicating important alterations in immunological parameters including TCD4+, TCD8+, Natural Killer cells, immunoglobulins and cytokines [5–10]. Considering the importance of inflammatory cytokines in many delayed complications of skin and lung injuries [11–13], this study has focused on the serum concentrations of the most important inflammatory cytokines including TNF, IL-1α, IL-1β and IL-1Ra.

Inflammatory cytokines such as TNF and IL-1 play a pivotal role in coordinating defense response to inflammation through interaction

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with their receptors. Tumor necrosis factor (TNF) is an important inflammatory cytokine in both acute and chronic inflammations which causes an inflammatory response through interaction with its receptors expressed on various cells such as endothelial cells. These cytokines increase vascular permeability allowing leukocyte access to the site of infection. In addition to this localized inflammatory response, systemic release of TNF may lead to septic shock and death [14]. Although interleukin-1 (IL-1) and TNF are structurally distinct and bind to different receptors, they have many closely related activities. IL-1 is responsible for many changes associated with the onset of a number of medical conditions and is involved in both acute-phase responses and chronic inflammatory conditions. IL-1α and IL-1β are two structurally related forms of IL-1 which bind to two types of receptors present on a variety of target cells. The type I receptor functions for signaling and type II as decoy, in addition to the soluble forms of receptors. IL-1 affects nearly every cell type, often in concert with other cytokines or other mediators and plays a role in development of disease and also in normal homeostasis [15,16]. This may include normal metabolism, hematopoiesis, renal and hepatic function, regulation of blood pressure, sleep, ACTH release and increased sodium excretion. IL-1 is produced by different cell types including macrophage-monocyte linage, epithelial cells and keratinocytes. During inflammation, injury, immunological challenge or infection, IL-1 is produced and contributes to the inflammatory response which may affect both acute and chronic diseases [17,18]. Interleukin-1 receptor antagonist (IL-1Ra) is also a member of the IL-1 family which has 30% amino acid sequence homology to IL-1β and binds to human IL-1 receptors types I and II and inhibits IL-1α and IL-1β by controlling their production and biological activity [12].

The aim of this study was to monitor the inflammatory cytokines; IL-1α, IL-1β, IL-1Ra and TNF in serum samples of the SM-exposed subjects, 20 years after exposure, in comparison to the control group.

2. Materials and methods

2.1. Study design and participants

The details of SICS study were reported previously [19]. In the present study there were 368 SM-exposed individuals that expose to SM 20 years ago and 126 unexposed participants as control group. The exposed group was classified in to hospitalized (H) and non-hospitalized (NH) groups according to clinical records and history of hospitalization at the exposure time. 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. Serum collection

Blood samples were allowed to clot at room temperature for 1 h and centrifuged for 20 min at 2000 g. Serum was gently removed, aliquoted and stored at −80 °C until used.

2.3. ELISA assay

Human IL-1α, IL-1β, IL-1Ra and TNF-α DuoSet® ELISA Development kits (R&D Systems) were used to measure the IL-1α, IL-1β, IL-1Ra and TNF-α levels in the sera. Primary antibody was mouse anti-human and biotinyalated goat anti-human was secondary antibody. All Standards 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, 1.5 mM KH_2PO_4. Rheumatoid factor (RF) Screen ELISA kit (DRG Instruments GmbH; Germany) was used to measure rheumatoid factor in the sera. CRP quantitative diagnostic kit was used to measure C-reactive protein (CRP) in sera by photometric method. In this method serum CRP makes complexes with polyclonal antibody against CRP. ELISA reader and washer were Stat-Fax 2100 and Stat-Fax 2600 (USA) respectively.

2.4. Statistical calculations

The data were presented as median and first and third quartiles (Q1–Q3). Statistical comparison among groups was performed using the Mann–Whitney test. Statistically significant value was defined as $p \leq 0.05$. Analyses of all data were performed with the SPSS 13 (SPSS Inc, Chicago, Illinois, USA).

3. Results

3.1. Serum levels of TNF

The results in Table 1 show the median of serum TNF levels in the exposed group which was about 11.11 pg/ml and was significantly ($p < 0.001$) lower than the control group (23.8 pg/ml). Among the exposed groups the lowest median of the serum TNF concentration was seen in the non-hospitalized (NH) group (9.4 pg/ml) although this value was not significantly lower than TNF levels in the hospitalized (H) group.

3.2. Serum levels of IL-1α

Median of the serum IL-1α levels in the exposed group was about 0.8 pg/ml which was significantly ($p < 0.001$) lower than the control group (1.9 pg/ml). There was no statistically significant difference between H and NH in the exposed group (Table 2).

3.3. Serum levels of IL-1β

Median of the serum IL-1β levels in the exposed group was about 1.7 pg/ml which was significantly ($p < 0.003$) lower than the control group (1.9 pg/ml) (Table 3). As the table shows the lowest serum IL-1β concentration value belongs to the non-hospitalized (NH) group (1.6 pg/ml) that is significantly lower than the IL-1β concentration of the hospitalized (H) group ($p < 0.002$).

Table 1
Serum TNF-α concentration (pg/ml) in the SM-exposed and control groups.

Study group		*N*	Median	Q1–Q3	Mean ± SEM	*p*-value[a]	*p*-value[b]
Control		126	23.79	0.533–95.44	31.86 ± 3.91		
Exposed	H	168	12.77	0.000–78.14	25.49 ± 3.78	**0.014**	0.227
	NH	200	9.38	0.000–82.81	19.59 ± 2.53	**0.001**	
	All exposed	368	11.11	0.000–79.17	22.28 ± 2.21	**0.001**	

The serum TNF-α level was assessed using ELISA method in all of the participants including the control and exposed groups. Exposed group (All exposed) was categorized into non-hospitalized (NH) and hospitalized (H) based on hospitalization in the time of exposure. A comparison was undertaken between each of the exposed groups with control group. Data was presented as median and the first and third quartiles (Q1–Q3).
SM: sulfur mustard, TNF-α: tumor necrosis factor-α.
[a] *p*-value: Comparison of the exposed, non-hospitalized and hospitalized groups with control group (Mann–Whitney).
[b] *p*-value: Comparison of hospitalized and non-hospitalized groups (Mann–Whitney).