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Fibrinogen and inflammatory cytokines in spontaneous sputum of sulfur-mustard-exposed civilians — Sardasht-Iran Cohort Study

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ABSTRACT

Sulfur mustard (SM) causes late complications in respiratory system of exposed individuals. In this preliminary study, the levels of IL-1α and β, TNF, IL-1Ra, IL-6 and fibrinogen in the spontaneous sputum of SM-exposed individuals were examined 20 years after exposure and the correlation with pulmonary function was tested.
The participants were categorized into two major subgroups (hospitalized and non-hospitalized) based on the severity of the clinical complications immediately after exposure. Every participant was visited by a physician; the respiratory functions were checked using spirometry and were categorized as normal, mild, moderate or severe pulmonary complications. The levels of cytokines in the sputum and serum samples were measured using ELISA method.
The mean values of TNF, IL-1α and IL-1β were 524.15, 115.15, 1951.33 pg/ml respectively, and the mean levels of IL-1Ra and IL-6 were 6410.52 and 124.44 pg/ml respectively; fibrinogen was 71.59 ng/ml and index of IL-Ra/IL-1β was 7.78. There was more TNF-α and IL-1β and less IL-1Ra and fibrinogen in the sputum of the hospitalized subgroup. The level of TNF-α and IL-1β also increased in moderate and severe pulmonary status comparing with the group with mild disorders, while fibrinogen was lower or decreased significantly in problematic patients. IL-1β and TNF showed positive correlation ($r = 0.5$, and $r = 0.59$, respectively); fibrinogen and IL1Ra/IL-1β have negative correlation with lung function according to the GOLD classification ($r = -0.4$, and $r = -0.61$, respectively).
It is concluded that sputum cytokines and fibrinogen, reflect the degree of the severity of airway inflammation and the cytokine levels in the sputum might be completely different from the serum fluctuations.

1. Introduction

Sulfur mustard (SM) causes various late complications in the lungs, eyes, and the skin of exposed individuals and the respiratory system is the most common affected organs and that is why the victims complain of numerous respiratory symptoms such as cough, sputum, hemoptysis, and chest pain [1,2]. In Iranian SM intoxicated people, the common lower respiratory diseases were chronic obstructive respiratory disease (84%), bronchiectasis (44.1%) and lung fibrosis (7.7%) [3]. One of the most studied tests in SM-exposed individuals is pulmonary function testing. Obstructive spirometric parameters including FEV (Forced Expiratory Volume in second 1), FVC (Forced Vital Capacity), FEV1, and FEV1/FVC (FEV1%) have all been affected in sulfur mustard intoxicated individuals [4–6].

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Cytokines especially inflammatory ones are considered as the main regulators of the above mentioned mediators in many other pulmonary complications [7], however the exact role of the cytokines in the long-term effect of sulfur mustard is not clearly defined. Many reports indicate that inflammatory cytokines play a central role in various pulmonary diseases [8–10].

According to the importance of locally produced cytokines, sputum is a mixture of mucus, cells and cellular products of the respiratory tract with about two liters a day might be used as a valuable non-invasive sample in pulmonary complications [11,12]. Sputum production is associated with different lung conditions and diseases including smoking, bronchitis, chronic obstructive pulmonary disease, asthma, acute obstructive airway disease and cystic fibrosis, although it seems that inflammation rather than any of the other changes that occur in damaged lung tissue is responsible for sputum production [13]. We recently reported that most of the inflammatory cytokines in the serum SM-exposed group of Sardasht-Iran Cohort Study (SICS) are lower than normal individuals [14,15], however, there is a another study which showed elevated inflammatory cytokines in the broncoalveolar lavage samples of SM-induced fibrosis [16]. To our knowledge there is no study on the sputum sample of SM-exposed individuals. Since the SM exposed population mostly complains about the respiratory problems, local samples such as sputum are a valuable non invasive sample which might give results different from systemic samples such as serum. The levels of inflammatory cytokines in induced sputum of normal lung are relatively lower (e.g. IL-1β: 62.4 pg/ml; IL-6: 25.5 pg/ml; TNFα: 0.0 pg/ml [17], or TNF between 0 and 15 pg/ml [18,19]). Fibrinogen as a biomarker in some pulmonary difficulties [20,21] is important in SM-exposed victims as well [22]. Besides the key role during blood clotting, fibrinogen plays a role in pathological processes such as atherosclerosis and a number of respiratory diseases [23]. Various inflammatory stimulators including infections induce lung alveolar epithelial cells to synthesize and secrete fibrinogen in a polarized manner [24,25] basolaterally which is consequently deposited into the extracellular matrix [26]. This extrahepatically produced matrix-bound fibrinogen deposition occurs independent of thrombin cleavage and could play a role in inflammation [25] and repair [27] e.g. fibrinogen (and also fibrin) serves as a ligand for integrins [28]. In the normal lung, the amount of alveolar or interstitial fibrinogen is insignificant [29]. On the other hand low levels of fibrinogen may also be an indicative of the activation of the system and as a result of faster consumption than synthesis. Inflammatory cytokines regulate fibrinolysis and, therefore suppression of cytokine signaling proteins inhibits pulmonary inflammation and fibrosis [30].

In this pilot study, the level of inflammatory cytokines, including interleukin-1 alpha and beta (IL-1α and β), tumor necrosis factor (TNF), interleukin-1 receptor antagonist (IL-1Ra), interleukin-6 (IL-6) and fibrinogen in the spontaneous sputum of SM-exposed individuals was examined and the correlation with pulmonary function was studied.

2. Materials and methods

2.1. Participants

The participants in this study were a subgroup of 40 male SM-exposed individuals in the Sardasht-Iran Cohort Study (SICS). Based on the documents in the medical records verified by the Medical Committee of the Foundation of Martyr and Veterans Affairs, the participants have been exposed to SM in June 1987. They had sputum spontaneously without any clinical symptoms of acute infection or febrile condition. In order to avoid any drug interference, those who were taking systemic immunosuppressive drugs were excluded. Other exclusion criteria were the history of systemic disease before exposure (based on medical records) or suffering from an acute infectious disease at the time of sampling. The mean age was 44.2 ± 9.9, and about 12% (4 of 34) were smokers and 88% were non-smokers.

The participants were categorized into two major subgroups based on the severity of the clinical problems at the time of exposure (1987); 1) hospitalized: patients with moderate to severe problems at the time of exposure who were hospitalized in the major cities in Iran or sent aboard for treatment, 2) non-hospitalized patients who had subclinical or mild problems at the time of exposure and were treated for acute effects as an outpatient [31,32].

2.2. Clinical evaluation

Every participant was visited by a physician and the respiratory functions were checked according to the American Thoracic Society's spirometric criteria (Chest 801 Spirometry), under the supervision of a trained nurse. A questionnaire on pulmonary symptoms (chronic cough, sputum, hemoptysis, and dyspnea) and pulmonary findings (crackles, rales, and wheezing) was completed for each patient based on examination by an internist.

The severity of the complications in the respiratory tract (pulmonary assessment), was graded as normal, mild, moderate, or severe based on the criteria set forth by the Medical Committee of the Foundation of Martyrs and Veterans Affairs by the experienced consultants of the research team [1]. The severity classification of pulmonary involvement was also done according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines.

Specimen collection as well as clinical evaluations was done in June 2007, 20 years after exposure.

2.3. Sputum and serum collection and processing

The volunteer patient was instructed to cough sputum into a sterile container when he felt that sputum might be present. Part of the specimen which was free from salivary contamination was separated, weighed, and diluted with fourfold volume of phosphate buffered saline (PBS). The specimen was vortexed for 10 min, centrifuged, aliquoted and kept frozen at −80 °C until use. Peripheral blood was drawn into Vacutainer tubes (BD Biosciences) and the serum was separated, aliquoted and kept at −80 °C until use.

2.4. ELISA measurements

Human TNF, IL-1α and IL-1β, IL-1Ra and IL-6 DuoSet® ELISA Development Kit (R&D Systems) and fibrinogen (AssayPro ELISA Kit) were used to measure the level of the mediators in the sera and sputum. This assay employs the quantitative sandwich enzyme immunoassay technique. ELISA reader and washer were Stat-Fax 2100 and Stat-Fax 2600 (USA) respectively. The detection limit of kits was between 1 and 3 pg/ml for cytokines and 3 ng/ml for fibrinogen.

2.5. Statistical analysis

Statistical comparison among the groups was performed using Mann–Whitney Test. Correlation between inflammatory mediators and pulmonary function parameters were computed with Spearman rank correlation coefficient. Difference of $p \leq 0.05$ was considered as statistically significant. Data are presented as mean (SD) and median (first and third quartiles). Analysis of the data was performed using SPSS software version 13.0 (Chicago, Illinois, USA).

3. Results

3.1. Inflammatory cytokines and fibrinogen in sputum of SM-exposed individuals

The concentrations of inflammatory cytokines and fibrinogen of SM-exposed individuals are introduced in Table 1. The mean value of TNF, IL-1α and IL-1β is 524.15, 115.15, 1951.33 pg/ml respectively,

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