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Serum soluble Fas ligand and nitric oxide in long-term pulmonary complications induced by sulfur mustard: Sardasht-Iran Cohort Study

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ABSTRACT

Sulfur mustard (SM) has short- and long-term toxicity against various organs including the respiratory system. However, the basic and molecular mechanisms of SM long-term toxicity have not clearly been defined. Thus, the aim of this study was to evaluate the association of soluble Fas ligand (sFasL) as well as nitric oxide (NO) serum levels with long-term pulmonary complications in a SM exposed population 20 years after SM exposure. In this historical cohort study 372 male SM exposed subjects and 128 age-matched unexposed controls were studied. Clinical evaluation and pulmonary function tests were carried out for all participants and serum concentrations of sFasL and NO measured. According to our results, the serum levels of sFasL and NO were not significantly different between the exposed and control groups. However, the serum levels of sFasL in the exposed group with pulmonary problems were significantly higher than their corresponding in the control group (116.711 ± 81.166 vs 86.027 ± 30.199 and $p = 0.028$). Furthermore a significant elevation in sFasL levels was found in the exposed subjects with pulmonary problems compared to those exposed participants without pulmonary problems (116.711 ± 81.166 vs 90.692 ± 57.853 and $p = 0.004$). Based on Global Initiative for chronic Obstructive Lung Disease (GOLD) classification analysis a positive correlation was observed between sFasL levels and pulmonary problems. There was also a significant negative correlation between sFasL and the white blood cell (WBC) count in the SM exposed cohort, but not in the control group. No significant association was shown between NO and pulmonary impairment in the SM exposed subjects. Thus, our results indicate that elevated serum levels of sFasL may be associated with progression of pulmonary diseases in the SM exposed subjects.

1. Introduction

Sulfur mustard (SM) induces acute, chronic and late injuries following exposure. Long-term clinical consequences of SM exposure target primarily the eyes, skin, respiratory and immune systems [1–4]. Previous studies revealed that respiratory problems are the most common long-term disorder among individuals exposed to SM [5,6]. Researchers have reported various types of respiratory manifestation in patients following SM toxicity, including pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), obliterative bronchiolitis, small airway disease and bronchiolitis [7,8]. There is little information and few studies that have identified the cellular and molecular mechanisms involved in these complications. Considering the importance of immune and inflammatory mediators in many delayed complications of lung injuries, in a comprehensive historical

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cohort study a large number of immunologic parameters and inflammatory markers was studied [1]. The present study is focused on the serum concentrations of the soluble Fas ligand (sFasL) and nitric oxide (NO).

Both FasL and NO are important molecules that play contradictory roles in inflammation and apoptosis. FasL (CD95L) is a 40 kD, 281-amino acid-containing type II transmembrane protein of the TNF family of death factors which is involved in regulating activation-induced cell death, establishment of immune privilege and tumor cell immune escape [9]. Fas ligand accumulates in soluble form at sites of tissue inflammation and has the potential to initiate apoptosis of leukocytes, epithelial cells, and other parenchymal cells [10]. Circulating levels of sFasL are reported to increase in some acute and chronic lung diseases including acute lung injury, acute respiratory distress syndrome, chronic obstructive pulmonary disease, asthma and fibrosing lung disease [11–13] and plays an important role in pathophysiology of these diseases.

Nitric oxide (NO) is a key physiological mediator in respiratory health and disease [14,15]. It has been shown that abnormal regulation of NO release is associated with the pathophysiology of almost all inflammatory diseases [16]. Currently research is focused on the therapeutic value of NO in different pulmonary inflammatory disorders [17]. It was reported that inducible nitric oxide synthase (iNOS) inhibitors have beneficial anti-inflammatory effects in a wide variety of acute and chronic animal models of inflammation including COPD [16].

Since there is no information on the role of FasL and NO in the long-term pulmonary complications of SM, we measured the serum concentrations of sFasL and NO in the exposed individuals, these results were then correlated with pulmonary complications 20 years after SM exposure.

2. Materials and methods

2.1. Study design and participants

The details of SICS study were previously reported [1]. In this study 372 SM exposed individuals that were exposed to SM 20 years ago and 128 unexposed participants as the control group were included. 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. Clinical evaluations

All participants were visited by clinicians. The respiratory signs and symptoms were evaluated by the experienced consultants of the research team. Then their respiratory functions were measured by spirometry [1,6]. Spirometries were done according to the American Thoracic Society Criteria [18], using a spirometry device (Chest 801 Spirometry) under the supervision of a trained nurse in three subsequent measurements and the best measurement was selected. The classification of the severity of pulmonary complications was done according to the Global Initiative for chronic Obstructive Lung Disease (GOLD) classification [6,19].

2.3. Serum collection

The blood samples were allowed to clot for 1 h at room temperature and centrifuged for 20 min at 2000×*g* (4 °C). Serum was collected, aliquoted and stored at −80 °C until laboratory assays.

2.4. Hematological methods

Blood count data was obtained from both the control and exposed groups within 1 h after collection of the blood samples. White blood cell (WBC) and Differential (Diff) tests were performed on each sample by an automatic cell counter (Sysmex, KX21N, Japan). Diff is carried out with K1000.

2.5. Serum soluble Fas ligand assessment

Human Fas Ligand DuoSet® ELISA Development Kit (R&D Systems) was used to measure Fas ligand in the sera. Primary antibody was mouse anti-human and biotinyalated 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 was 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. This kit is capable of detecting the secretory form of sFasL (not the proteolytic cleaved membrane form). ELISA reader and washer were Stat-Fax 2100 and Stat-Fax 2600 respectively.

2.6. Nitrite assay

Nitrite level in the serum was determined by a method based on the Griess reaction using a Kayman product kit (USA). A total of 100 µL of the sample was mixed with 100 µL of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylenediamide dihydrochloride in water) and incubated at room temperature for 10 min followed by measuring the absorbance in a plate reader at 540 nm (Stat-Fax 2100). Nitrite concentration in the samples was determined from a standard curve generated by different concentrations of sodium nitrite [20].

2.7. Statistical analysis

Data was presented as mean ± standard deviation. Comparison of sFasL among groups was performed using *t*-test. Correlation between sFasL and the pulmonary function parameters was computed with Pearson correlation coefficient. $p \leq 0.05$ was considered as a statistically significant level. Analyses were performed with the SPSS software version 13 (SPSS Inc. Chicago, Illinois, USA).

3. Results

3.1. Circulating levels of sFasL and NO

Data presented in Table 1 shows that there was no significant differences in sFasL and NO serum levels between the SM exposed and

Table 1
Comparison of the serum levels of sFasL and NO between the SM exposed and the control groups.

		Control			Exposed			*p*-value
		N	Mean	SD	*N*	Mean	SD	
sFasL	All participants	127	97.324	47.400	370	93.703	60.111	0.610
	Non-smokers	98	97.380	46.923	297	94.691	60.616	0.689
NO	All participants	127	1422.6	563.5	370	1509.2	558.9	0.135
	Non-smokers	98	1451.1	545.0	297	1548.7	579.7	0.145

The serum levels of sFasL and NO were assessed using ELISA method, and a comparison was undertaken between the control and exposed groups (for all participants or Non-smokers participants). *p*-value: comparison of exposed with control groups (*t*-test), sFasL: soluble Fas ligand, and NO: nitric oxide.