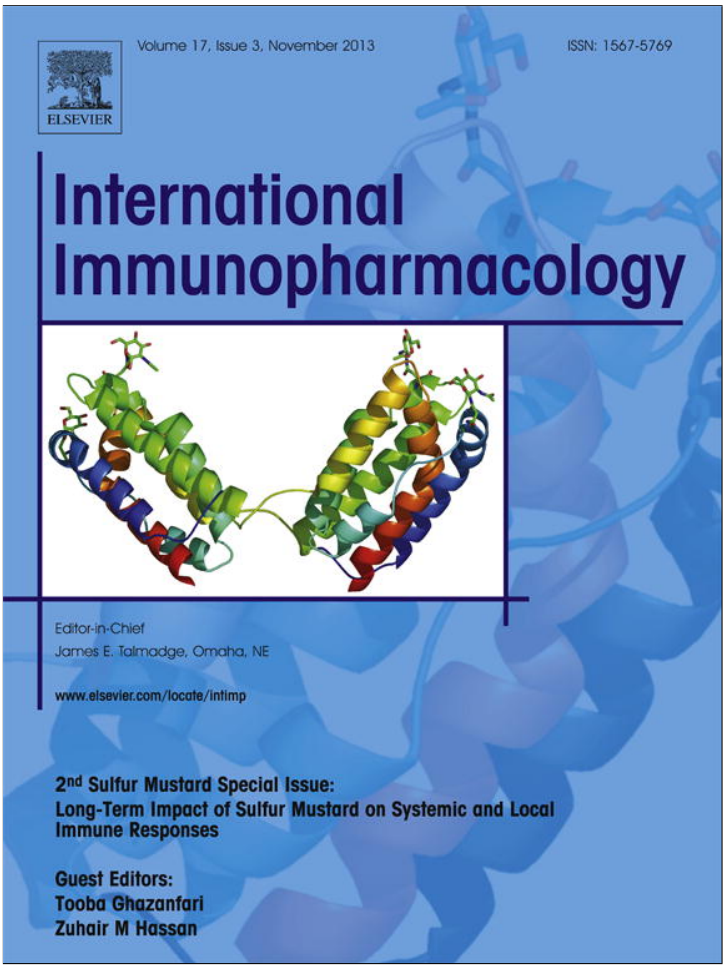

International Immunopharmacology 17 (2013) 968–973

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

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

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ARTICLE INFO

Article history:
Received 28 December 2011
Received in revised form 5 December 2012
Accepted 27 December 2012
Available online 29 January 2013

Keywords:
Sulfur mustard
Sputum
Cytokines
Spirometry
Pulmonary complications

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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http://dx.doi.org/10.1016/j.intimp.2012.12.024

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,

Table 1
The concentrations of cytokines in sputum of SM-exposed civilians of Sardasht.

	N	Mean ± SD	Median	Q1–Q3[a]
TNF-α (pg/ml)	34	524.15 ± 788.79	145.87	63.22–633.70
IL-1α (pg/ml)	34	115.15 ± 71.4	97.96	57.81–165.60
IL-1β (pg/ml)	34	1951.2 ± 1289.33	1816.50	738.6–3182.0
IL-1Ra (pg/ml)	34	6410.52 ± 2665.98	7521.25	468–840
IL-1Ra/IL-1β	34	7.78 ± 11.35	3.02	2.23–8.10
IL-6 (pg/ml)	34	124.44 ± 251.02	34.85	9.67–73.96
Fibrinogen (ng/ml)	34	71.59 ± 40.82	75.43	27.12–111.08

The participant was instructed to cough sputum into a sterile container when he felt that sputum might be present. Part of the specimen, was separated, and diluted with fourfold volume of PBS. Cytokines and fibrinogen were measured using ELISA technique.IL: interleukin, TNF: tumor necrosis factor, SM: sulfur mustard.
[a] Q1–Q3: first and third quartiles.

IL-1Ra and IL-6 are 6410.52 and 124.44 pg/ml respectively; fibrinogen is 71.59 ng/ml and the index of IL-Ra/IL-1β is 7.78.

3.2. Sputum cytokines and fibrinogen in subgroups of SM-exposed individuals

SM-exposed individuals were divided into hospitalized and non-hospitalized subgroups according to the severity of injuries at the time of exposure. As shown in Table 2, the values of TNF-α, IL-1β, IL-1Ra and fibrinogen are significantly different between the two subgroups. There are significantly more TNF-α and IL-1β and significantly less IL-1Ra and fibrinogen in the sputum of the hospitalized patients when compared to the non-hospitalized group. In addition, the index of IL-1Ra/IL-1β is significantly ($p = 0.008$) lower in the hospitalized group in comparison with the non-hospitalized group. No statistically significant difference was seen in IL-1α and IL-6 levels between the two groups.

Individuals were subdivided into 3 subgroups (normal, mild and moderate-severe) by pulmonary function test based on clinicians' evaluation and spirometry assessment (at the time of sampling i.e. 20 years after SM exposure). The level of cytokines in each subgroup is indicated in Table 3.

The most significant elevated cytokine levels in the sputum of moderate–severe conditions were TNF and IL-1β with the median about 9.5 and 2.3 times (respectively) higher than the normal pulmonary function conditions. Fibrinogen was significantly lower (about 3 folds) in problematic individuals. IL-1Ra was lower in moderate–severe conditions, according to GOLD spirometry classification.

There was no significant difference between the cytokine levels in individuals with clinical symptoms and signs including chronic cough, hemoptesia, dyspnea, chest pain and pulmonary auscultation (including rale, wheezing or crackle) except for sputum level of IL-1β which was significantly lower in patients with chest pain (1119.0 pg/ml, $n = 19$) in comparison to patients without chest pain (3079.0 pg/ml, $n = 9$) ($p < 0.037$).

3.3. Correlations of sputum cytokines and fibrinogen with spirometric parameters

TNF showed a negative correlation with FEV1, while IL-1Ra showed a positive correlation, although only TNF showed a positive and significant correlation with spirometry GOLD. IL-1β levels displayed a negative correlation with FEV1/FVC and positive correlation when spirometry GOLD was used. Fibrinogen showed a negative correlation with spirometry GOLD as well (Table 4).

3.4. Strong correlations between sputum cytokines and fibrinogen

As it is shown in Table 5, there are strong correlations (both negative and positive) between inflammatory cytokines and fibrinogen as well. Sputum levels of IL-1α, IL-1β, and TNF showed significant

Table 2
The concentrations of cytokines in sputum of SM-exposed civilians of Sardasht (hospitalized vs. not hospitalized)[a].

Study group	N	Mean	SD	p-value[1]	Median	Q1	Q3	p-value[2]
	TNF-α (pg/ml)							
Not hospitalized	18	167.967	190.188	**0.004**	86.075	43.120	194.350	0.003
Hospitalized	16	924.849	1002.16		679.600	133.525	1486.250	
	IL-1 α (pg/ml)							
Not hospitalized	18	106.560	61.439	0.465	91.580	48.670	165.600	0.506
Hospitalized	16	124.822	82.151		109.750	59.755	188.500	
	IL-1 β (pg/ml)							
Not hospitalized	18	1340.106	1118.26	**0.002**	967.425	395.30	1950.0	0.003
Hospitalized	16	2638.675	1134.77		3153.250	1796.25	3393.0	
	IL-1 Ra (pg/ml)							
Not hospitalized	18	7383.444	2185.84	**0.022**	8247.500	7568.000	8712.500	0.001
Hospitalized	16	5315.988	2794.22		6356.500	2603.750	7516.000	
	IL-1Ra/IL-1β							
Not hospitalized	18	12.506	14.013	**0.008**	6.768	3.317	13.276	<0.001
Hospitalized	16	2.465	1.958		2.265	1.154	2.578	
	IL-6 (pg/ml)							
Not hospitalized	18	98.847	195.881	0.537	51.665	14.670	73.965	0.347
Hospitalized	16	153.238	305.724		27.915	7.126	77.063	
	Fibrinogen (ng/ml)							
Not hospitalized	18	88.266	38.202	**0.011**	89.980	74.460	113.960	0.018
Hospitalized	16	52.688	36.085		27.760	24.380	76.940	

p-value[1]: T-test comparison between hospitalized and non-hospitalized.
p-value[2]: Mann–Whitney test comparison between hospitalized and non-hospitalized.
[a] The participants were categorized into two major subgroups based on the severity of the clinical problems at the time of exposure: 1) hospitalized: victims who had moderate to severe problems at the time of exposure and were hospitalized, 2) non-hospitalized: patients who had subclinical or mild problems at the time of exposure and were treated for acute effects as outpatient. IL: interleukin, TNF: tumor necrosis factor, SM: sulfur mustard.

Table 3
The levels of inflammatory cytokines and fibrinogen in sputum of Sardasht SM-exposed individuals having spontaneous sputum based on pulmonary function.

	Pulmonary function[a]		N	Median	Q1	Q3	p-value
TNF-α (pg/ml)	Pulmonary assessment	Normal	15	130.70	77.64	536.69	
		Mild	7	63.22	33.37	149.75	0.162
		Moderate-Severe	6	1246.25	220.20	2316.00	**0.008**
	Spirometry GOLD	Without problem	19	82.68	44.66	142.00	**0.000**
		With lung problem	12	910.35	214.95	1674.50	
IL-1α (pg/ml)	Pulmonary assessment	Normal	15	112.100	70.620	194.200	
		Mild	7	57.810	42.600	68.980	**0.009**
		Moderate-Severe	6	139.355	101.400	223.400	0.470
	Spirometry GOLD	Without problem	19	80.370	45.135	153.600	0.110
		With lung problem	12	120.098	66.288	230.100	
IL-1β (pg/ml)	Pulmonary assessment	Normal	15	1485.0	678.1	3225.0	
		Mild	7	873.4	290.7	2444.5	0.142
		Moderate-Severe	6	3393.0	3168.0	3830.0	**0.023**
	Spirometry GOLD	Without problem	19	1119.0	395.3	2694.0	**0.005**
		With lung problem	12	3153.3	1796.3	3542.0	
IL-1Ra (pg/ml)	Pulmonary assessment	Normal	15	8042.0	6157.0	8574.0	
		Mild	7	6875.0	3129.5	7727.0	0.237
		Moderate-Severe	6	3943.8	2001.0	7181.0	0.095
	Spirometry GOLD	Without problem	19	7788.0	6875.0	8533.0	**0.048**
		With lung problem	12	5419.0	2565.3	7351.3	
IL-6 (pg/ml)	Pulmonary assessment	Normal	15	34.610	11.656	137.400	
		Mild	7	55.540	35.090	73.965	0.630
		Moderate-Severe	6	8.646	3.486	33.755	0.066
	Spirometry GOLD	Without problem	19	49.500	11.656	137.400	0.589
		With lung problem	12	28.608	8.646	67.433	
Fibrinogen (ng/ml)	Pulmonary assessment	Normal	17	70.000	43.560	79.880	
		Mild	9	84.800	81.080	126.440	**0.013**
		Moderate-Severe	5	24.380	20.540	27.360	**0.009**
	Spirometry GOLD	Without problem	23	79.880	45.420	107.950	**0.023**
		With lung problem	11	27.360	22.300	65.240	
IL1Ra/IL1β	Pulmonary assessment	Normal	15	5.407	2.298	10.043	
		Mild	7	3.583	2.603	17.392	0.581
		Moderate-Severe	6	1.154	0.806	2.092	**0.005**
	Spirometry GOLD	Without problem	19	5.676	2.612	11.204	<**0.001**
		With lung problem	12	2.116	0.819	2.578	

Mann–Whitney test p-value: Mann–Whitney test comparison with normal. Bold data shows significant differences with p value <0.05.

[a] All participants were visited by clinicians and their respiratory functions were measured according to the American Thoracic Society's spirometric criteria. The severity of 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. The classification of pulmonary involvement was also done according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines. IL: interleukin, TNF: tumor necrosis factor, SM: sulfur mustard.

positive correlations with each other, but not with IL-6 or IL-1Ra. Fibrinogen showed a negative correlation with TNF and IL-1β levels and a positive correlation with IL-6 and IL-1Ra. However, there is no significant correlation between fibrinogen and IL-1α.

Table 4
The correlations between spirometric parameters and sputum cytokines in SM-exposed individuals having spontaneous sputum.

		FVC %	FEV1 %	FEV1/FVC %	Spirometry GOLD
TNF-alpha	r	−0.167	−0.395	−0.339	0.598
	p	0.368	**0.031**	0.156	**0.000**
IL-1α	r	0.115	−0.028	−0.270	0.273
	p	0.538	0.884	0.263	0.138
IL-1β	r	−0.118	−0.312	−0.526	0.504
	p	0.527	0.093	**0.021**	**0.004**
IL-1Ra	r	0.348	0.364	0.181	−0.333
	p	0.055	**0.048**	0.459	0.067
IL1Ra/IL-1β	r	0.315	0.441	0.505	−0.611
	p	0.085	**0.015**	**0.027**	<**0.001**
IL-6	r	0.101	0.082	0.172	−0.131
	p	0.588	0.668	0.482	0.483
Fibrinogen	r	0.186	0.240	0.140	−0.407
	p	0.293	0.179	0.514	**0.017**

Correlation between inflammatory mediators and pulmonary function parameters were computed with Spearman rank correlation coefficient. IL: interleukin, TNF: tumor necrosis factor, SM: sulfur mustard. FVC: Forced Vital Capacity; FEV1: Forced Expiratory Volume in second 1. Bold data shows significant differences with p value <0.05.

3.5. Correlation between serum and sputum concentration of cytokines and fibrinogen in SM-exposed individuals

In order to explore any possible correlation between serum concentration of these cytokines and the situation of pulmonary function or the intensity of the SM exposure, as it is shown in Table 6, although the total values were previously reported [14], here the serum values and sputum concentrations are compared.

Table 5
The correlations between sputum cytokines and fibrinogen.

		Sputum					
		IL-α	IL-1β	IL-1Ra	IL-1Ra/IL1β	IL-6	FBG
TNF-α	r	0.366	**0.650**	−0.293	**−0.653**	−0.077	**−0.622**
	p	0.033	**0.000**	0.093	**0.000**	0.666	**0.000**
IL-1α sputum	r	1	**0.511**	0.249	−0.282	−0.134	−0.284
	p		**0.002**	0.156	0.107	0.451	0.115
IL-1β sputum	r		1	−0.142	**−0.814**	0.033	**−0.508**
	p			0.423	**0.000**	0.854	**0.003**
IL-1Ra sputum	r			1	0.561	0.191	0.399
	p				0.001	0.278	0.024
IL-1Ra/IL-1β sputum	r				1	0.162	**0.621**
	p					0.359	**0.000**
IL-6 Sputum	r					1	0.397
	p						0.025

Bold data shows significant differences with p value <0.05.
r: Spearman rank correlation coefficient.
IL: interleukin, TNF: tumor necrosis factor, SM: sulfur mustard.

Table 6
The correlations between sputum and serum cytokines in SM-exposed individuals having spontaneous sputum.

Sputum							
Serum		IL-1α	IL-1β	IL-1 Ra	IL-6	TNF-α	FBG
IL-1α	r	0.130	**0.414**	−0.360	0.055	0.325	−0.094
	p	0.465	**0.015**	**0.037**	0.757	0.061	0.608
IL-1β	r	0.188	0.049	−0.074	0.152	0.148	−0.040
	p	0.286	0.784	0.676	0.390	0.405	0.830
IL-1 Ra	r	0.014	0.101	−0.223	−0.206	0.174	−0.088
	p	0.936	0.568	0.205	0.243	0.326	0.632
IL-6	r	0.095	0.119	−0.291	0.093	**0.396**	−0.216
	p	0.594	0.503	0.095	0.600	**0.020**	0.234
TNF-α	r	0.260	**0.417**	−0.266	0.128	0.335	−0.081
	p	0.137	**0.014**	0.129	0.469	0.053	0.659
IL-1Ra/IL-1β	r	−0.107	−0.024	−0.067	−0.337	−0.022	−0.006
	p	0.548	0.893	0.708	0.051	0.900	0.973

Bold data shows significant differences with p value <0.05.
r: Spearman rank correlation coefficient.
IL: interleukin, TNF: tumor necrosis factor, SM: sulfur mustard.

Positive correlation between the serum and the sputum level of cytokines show only for TNF, however serum level of TNF has positive correlation with the sputum level of IL-1β as well. Serum level of IL-1α showed also a positive correlation with sputum level of IL-1β and a negative correlation with sputum IL-R1a. A positive correlation was observed between the serum level of IL-6 and TNF in the sputum.

4. Discussion

Sputum production is associated with various lung diseases and could be used as a non-invasive valuable sample for evaluating locally produced cytokines in the respiratory tract. It is postulated that the main trigger of sputum production is inflammation rather than the disease [13]. The reported levels of inflammatory cytokines in induced sputum of normal lung is lower than normal e.g. IL-1β: 62.4 pg/ml; IL-6: 25.5 pg/ml; TNFα: 0.0 pg/ml [17], or between 0 and 15 pg/ml [18,19]. In this study, the levels of inflammatory cytokines and fibrinogen in the spontaneous sputum of Sardasht sulfur mustard exposed victims were examined in order to evaluate their local production.

The mean concentrations of TNF-α, IL-1α and IL-1β in sputum of SM-exposed individuals were 524.1, 115.15 1951.3 pg/ml respectively, IL-1Ra and IL-6 were 6410.5 and 124.4 pg/ml respectively; and the index of IL-1Ra/IL-1β was 7.8 (Table 1). The level of inflammatory cytokines especially TNF and IL-1β in SM-exposed individuals with spontaneous sputum, were augmented significantly.

In each case, the results were also shown for hospitalized (i.e. those who at the time of exposure 20 years ago had more serious injuries and were hospitalized) and non-hospitalized victims. As observed, the values of TNF-α, IL-1β and IL-1Ra are significantly different between the two subgroups (Table 2). There are significantly higher levels of TNF-α and IL-1β and significantly lower levels of IL-1Ra in the sputum of the hospitalized ones. The index of IL-1Ra/IL-1β was significantly lower in the hospitalized group compared with the non-hospitalized group (2.5 versus 12.5).

There are reports indicating increased levels of interleukin (IL)-6, IL-1beta, tumor necrosis factor-alpha (TNF-alpha) and IL-8 in sputum of COPD patients [8]. The mean levels of IL-6, IL-8 and TNF-α in induced sputum were found to be higher in severe–very severe COPD than in mild–moderate COPD. A significant correlation was observed between the IL-6 value and FEV1, FEV1/FVC and disease duration [7]. Although according to Moermans et al., sputum cell cultures in COPD produced significantly less or no IL-6, IL-10, and TNF-α compared to the healthy subjects [33].

In our study the sputum level of TNF and IL-1β showed a strong positive correlation with moderate–severe lung problems (Table 3). It may be expected that the amount of inflammatory cytokines increases in parallel with severity of lung problems i.e. in individuals with severe pulmonary problems (clinically evaluated) more than mild and in mild more than non-problematic ones. However a reduction is observed in mild pulmonary problems even in comparison with non-problematic individuals which was statistically significant for IL-1α (112.1, 57.8 and 139.3 pg/ml for non-, mild and severe pulmonary problems respectively). It seems that an inclination towards non- or anti-inflammatory conditions is prevalent among mild-pulmonary problems which might be interpreted as a defense mechanism against high inflammatory conditions induced by SM, in seriously injured individuals, this occurred immediately. Similar results have also been observed previously [14].

The level of anti-inflammatory cytokine IL-1Ra however is higher in the sputum of individuals without lung problems (according to spirometry GOLD) i.e. 7788.0 versus 5419.0 pg/ml. The index of IL-1Ra/IL-1 β shows the same result as well, i.e. 5.7 in non-problematic function-test versus 2.1 in problematic victims.

The most significant positive correlations between cytokines and spirometry GOLD was observed in TNF and IL-1β ($r=0.598$ $p<0.000$ and $r=0.504$ $p<0.004$ respectively). However, IL1Ra/IL-1β and fibrinogen showed the most significant negative correlation with spirometry GOLD ($r=-0.611$ $p<0.001$ and $r=-0.407$ $p<0.017$ respectively) (Table 4).

Plasma fibrinogen with a normal level of about 1.5–2.77 g/L in serum which increases in inflammation as an acute phase protein [20,21,34–36] and could be considered as a biomarker in some pulmonary complications [20,21]. This is likely to be the first COPD biomarker [35] and is important in SM-exposed victims [20]. According to Dahl et al., individuals with baseline plasma fibrinogen of more than 3.3 g/L versus less than 2.7 g/L showed reduced lung function and increased cumulative incidence of COPD hospitalization [37].

It is reported that in the normal lungs, the amount of alveolar or interstitial fibrinogen is insignificant [29] and augmentation of the level in respiratory tract could be a marker of plasma exudation [38,39]. Besides various inflammatory stimulators may induce lung alveolar epithelial cells to synthesize and secrete fibrinogen in a polarized manner [25,26]. It has been reported that the levels of fibrinogen, plasminogen and plasminogen activator inhibitor (PAI)-1 in hypertonic saline-induced sputum of moderate or severe asthmatic patients were higher than in mild ones and it has been concluded that acute exacerbation of moderate asthma is associated with a shift to a profibrinogenic possibly antifibrinolytic, environment in the airways. [40]. In a proteomic study of bronchoalveolar lavage fluid of sulfur mustard-exposed patients with lung disease, a significant increase in fibrinogen – among other parameters – has been observed, especially in moderate and severe lung disease patients [20].

In our study, the amount of sputum fibrinogen was about 71.6 ng/ml in all SM-exposed participants, there was significantly ($p<0.011$) less fibrinogen (52.7 ng/ml) in the sputum of hospitalized individuals in comparison to non-hospitalized individuals (88.3 ng/ml) (Table 2).

There was a negative correlation between sputum fibrinogen and TNF, IL-1α and IL-1β and a positive correlation with IL-6 and IL-1Ra (Table 5) which may emphasize that sputum fibrinogen correlates with less local inflammatory conditions in this study. In addition, the fact that it has no correlation with any one of the above-mentioned cytokines in the serum confirms the premise (Table 6) which suggests the differential regulation of the local level of fibrinogen. There was clearly less sputum fibrinogen in individuals with lung problems (according to clinical examination at the time of sputum sampling) i.e. 27.4 ng/ml in problematic versus 79.9 ng/ml and also a significant negative correlation between the sputum level of fibrinogen and spirometry GOLD (Table 4). There are studies which showed an increase in fibrinogen level in plasma and a few also in sputum or BAL with lung and other inflammatory diseases [38,39]. Here a significant increase in fibrinogen level which was less in problematic individuals than in non-problematic ones.

It may be attributed to local activation of the fibrinolytic system i.e. consumption occurs faster than synthesis. According to the findings

which showed that elastase is significantly increased in the sputum of SM-exposed victims and the important role in degradation of fibrinogen [41] which has a key task in the extravascular space [42], it could be suggested that the low levels of fibrinogen might be the result of enhanced elastase activity. The inhibition of fibrinolytic activity may play a role in the pathogenesis of lung fibrosis [43].

A significant correlation between sputum IL-6 and fibrinogen was seen but there was no correlation between sputum IL-6 and pulmonary function or serum IL-6 and sputum fibrinogen, although IL-6 is one of the most important stimulators of fibrinogen production from the liver.

In this study, important changes in inflammatory cytokines produced in the respiratory tract were observed. The current study also demonstrated significant correlations between fibrinogen, inflammatory cytokines and pulmonary function, e.g. TNF and fibrinogen, which can reflect the degree of the severity of airway inflammation. It can also be concluded that sputum is a valuable specimen for evaluating respiratory system status. Therefore, immunomodulation could be considered as a new and crucial approach in the treatment of SM exposed patients in the long term. Our study provides insight into the therapeutic strategies taken towards SM-exposed victims, particularly the long-term follow-up of their health conditions.

Declaration of interest

The authors report no conflict of interest in this study.

Acknowledgment

This study was carried out by the Immunoregulation Research Center of Shahed University and Janbazan Medical and Engineering Research Center (JMERC) and financially supported by the Iranian Foundation of Martyr and Veterans Affairs and the Ministry of Health and Medical Education. The authors would like to kindly thank all the participants who took part in this study.

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