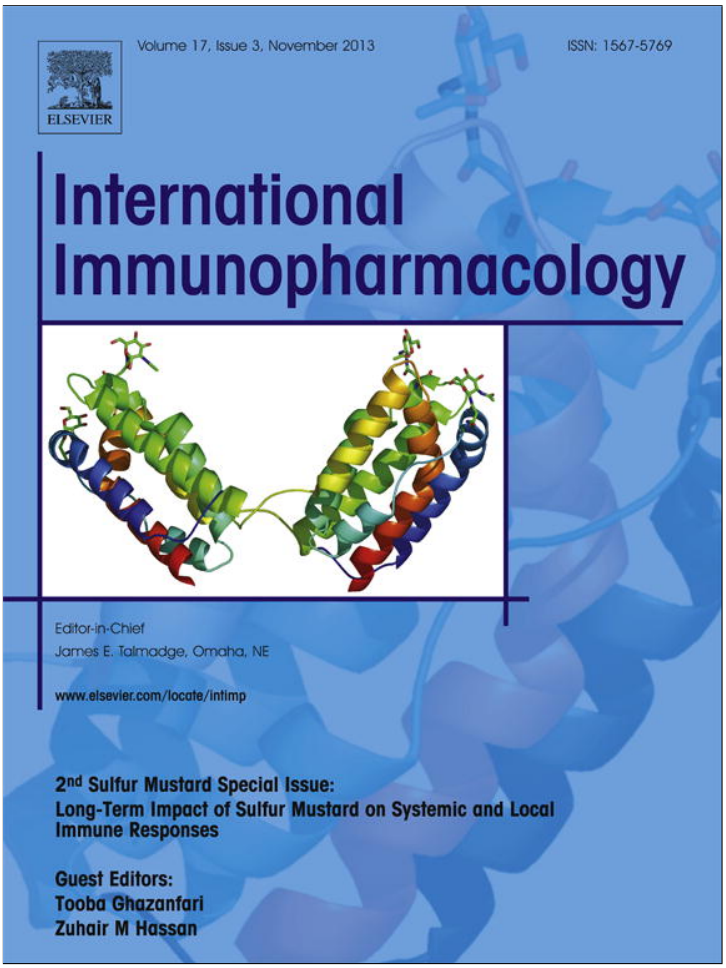

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Chemokines, MMP-9 and PMN elastase in spontaneous sputum of sulfur mustard exposed civilians: Sardasht-Iran Cohort Study

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ABSTRACT

Chemokines play an important role in acute and chronic pulmonary diseases. The aim of this study was to evaluate the levels of chemokines, MMP-9, and PMN elastase in spontaneous sputum and serum of patients 20 years after SM exposure. In context of Sardasht-Iran Cohort Study (SICS) 40 male volunteers with a history of SM exposure in June 1987 and complain of excessive sputum were recruited. The volunteers were clinically examined and their history was collected by internists. Sputum and serum levels of IL-8, fractalkine, MCP-1, RANTES, MMP-9, and PMN elastase were measured using ELISA kits (R&D System). Spirometries were performed on all the participants. Sputum level of fractalkine was significantly lower in the hospitalized group (N = 16, Median = 1.05; IQR = 0.41–2.62) than non-hospitalized group (N = 18, 4.031; IQR = 0.947–8.203) (p = 0.042). However, serum levels of fractalkine were higher in the hospitalized group (Mean ± SD = 2.08 ± 5.09) than in the non-hospitalized (Mean ± SD = 0.53 ± 0.87) group (T-test, p = 0.03). Serum levels of PMN-elastase were also higher in the hospitalized group (Mean ± SD; 64,794.43 ± 26,820.08) than in the non-hospitalized group (Mean ± SD = 44,049.33 ± 17,675.85) (p = 0.017). There was no relationship between the cytokines and the studied factors in sputum and the GOLD classification, but the serum levels of fractalkine and MMP-9 were significantly higher in the more severe (grades 3–4) group. There was no significant correlation between sputum and serum levels of measured inflammatory mediators and pulmonary complications in the patients who were exposed to SM 20 years earlier. Pathophysiologic process involved in SM induced pulmonary problems might be different from those in other chronic pulmonary diseases such as COPD and asthma.

1. Introduction

The lungs, eyes and skin are the organs mostly affected by SM toxicity [1,2]. Of these, the lungs are the site of the most common and most disabling long-term complications. The mechanism(s) of mustard gas toxicity is not well known. To understand more its mechanisms, a comprehensive historical cohort study was recently established in a civilian SM-exposed population in Sardasht, Iran which is defined as the Sardasht-Iran Cohort Study (SICS) [3]. In these patients, dyspnea was the most common symptom. Chronic cough, sputum, hemoptysis, and chest pain were also common pulmonary findings in this population [4]. A previous study by the same group showed that the serum levels of pro-inflammatory cytokines IL-1a, IL-1b, IL-1Ra and TNF were significantly lower in the SM-exposed group [5]. However, the serum levels of MCP1/CCL2

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were significantly higher and the level of MMP9 and fractalkine showed no change in the cohort [6].

In general terms, chemokines have been shown to play an important role in acute and chronic pulmonary diseases [7]. IL-8/CXCL8 is a chemokine secreted from various sources in response to different stimuli and participate in, acute inflammation due to potent actions on neutrophils. IL-8/CXCL8 plays a role in the pathogenesis of chronic pulmonary diseases such as COPD, pulmonary fibrosis and asthma [8]. RANTES (Regulated upon Activation, Normal T-cell Expressed, and Secreted) is involved in inducing fibrous airway obliteration (FAO) in transplanted lungs and as such local administration of anti-RANTES might be a therapeutic option for bronchiolitis obliterans (BO) following lung transplantation [9]. Monocyte chemoattractant protein-1 (MCP-1) belongs to the C–C chemotactic cytokine family and plays certain role in the pathogenesis of fibrotic lung diseases. MCP-1 recruits monocytes, memory T cells, and dendritic cells to the site of tissue injury, infection, and inflammation. Increased expression of MCP-1 in the lung tissues of patients with idiopathic pulmonary fibrosis (IPF) has been reported [10]. Fractalkine (CX3CL1) is the only member of the CX3C chemokine family which exists as a membrane-bound and soluble form and interacts with cells expressing its specific receptor (CX3CR1). Fractalkine has chemotactic, adhesive, and cytotoxic activities. It has been shown that this chemokine plays certain roles in the pathogenesis of various lung diseases [11]. Matrix metalloproteinase-9 (MMP-9) is responsible for degradation of extracellular matrix (ECM) components including basement membrane collagen. Low levels of MMP-9 have been reported in normal lungs but it has been found to increase significantly in several lung diseases including asthma, idiopathic pulmonary fibrosis (IPF), and chronic obstructive pulmonary disease (COPD). Specific tissue inhibitors of matrix metalloproteinase (TIMP) regulate the potent proteolytic activities of MMPs [12,13]. Neutrophil elastase is a protease which is involved in tissue destruction and inflammation and plays an important role in the pathogenesis of various lung diseases including emphysema, chronic obstructive pulmonary disease, cystic fibrosis, and adult respiratory distress syndrome [14,15].

A few studies have evaluated the role of chemokines in SM-related pulmonary complications. Emad et al. reported the elevated levels of IL-8, IL-1b, IL-6, TNF-a, and IL-12 in the bronchoalveolar lavage fluid (BALF) of patients with SM-induced pulmonary fibrosis [16,17]. Previous studies of the same group of SICS also showed that the serum levels of IL-8, IL-6 and RANTES/CCL5 were significantly decreased in the SM-exposed group. No significant difference was observed in the serum levels of MCP-1/CCL2 or fractalkine/CX3CL1 between the patients with SM-induced pulmonary complications and the control groups. No significant association was seen between the serum levels of IL-8 and IL-6 and pulmonary symptoms, signs, and spirometry parameters, but the serum levels of RANTES/CCL5 were significantly decreased with lower FEV1/FVC [6,18]. Since local levels of chemokines, MMPs, and other inflammatory mediators are more important than circulatory levels in pulmonary disease, and the sputum collection is less-invasive than bronchoscopy for BALF, in the current study the sputum levels of IL-8/CXCL8, RANTES, MCP-1, fractalkine, MMP-9, and PMN-elastase were evaluated and compared with the serum levels. The relationship between the inflammatory mediators and pulmonary symptoms, signs and function was assessed.

2. Material and methods

2.1. Participants

The participants in the present study were a subgroup of 40 male SM-exposed individuals in the Sardasht-Iran Cohort Study (SICS). Based on the medical record documents which are verified by the Medical Committee of the Foundation of Martyr and Veterans Affairs, the participants have been exposed to SM in 1987. The volunteers had spontaneous sputum without any clinical symptom of acute infection or febrile condition. Patients with history of immunosuppressive drug consumption in the past month or history of chronic inflammatory diseases were excluded. Other criteria for patient selection have been previously reported in the SICS methods [3].

Participants were classified into two groups; hospitalized and non-hospitalized based on the hospitalization and severity of the clinical problems at the time of exposure. The investigators used hospitalization as an index for the severity of exposure and pulmonary involvement. The age range of the participants was 20–60 years (Mean of 44.3 ± 9.8) for all the participants and 46.9 ± 7.6 for hospitalized vs. 41.7 ± 11.3 years for non-hospitalized, there was no significant difference between the two groups (Chi square = 0.111).

2.2. Ethical considerations

The study was approved by the ethical committee of the Board of Research Ethics of Janbazan Medical and Engineering Research Center (JMERC), the Board of Research of Ministry of Health, and Shahed University. Individuals who wish to participate and sign an informed consent were recruited.

2.3. Clinical evaluation

Internists examined the study participants and completed a questionnaire surveying pulmonary symptoms (chronic cough, sputum, hemoptysis, and dyspnea) and pulmonary findings (fine crackles, coarse crackles, and wheezing). Chronic cough was defined as persisting cough for more than 3 weeks. Three subsequent spirometry measurements (Chest 801 Spirometry) were performed on all participants according to the American Thoracic Society Criteria under supervision of a trained nurse. The suitable measurement was selected for data collection. The classification of severity of pulmonary involvement was done according to the Global initiative for chronic Obstructive Lung Disease (GOLD).

2.4. Sputum collection and processing

Participants were instructed to cough sputum into a sterile container if they felt that sputum might be present. After the removal of the saliva, the specimen was weighed and a 4-fold volume of phosphate buffered saline (PBS) was added to disperse the sputum. After 10 min of vortex, each sample was centrifuged, aliquoted, and kept frozen at −70 °C for later cytokine analysis.

2.5. Serum collection

Peripheral blood sample was drawn into Vacutainer tubes (BD Biosciences). The serum was separated for 20 min by centrifugation at 2000 ×*g* (4 °C), aliquoted, labeled and kept frozen at −80 °C until laboratory measurements.

Table 1
Comparison of clinical findings between hospitalized and non-hospitalized groups exposed to sulfur mustard in 1987 Sardasht, Iran.

Clinical findings	Hospitalized (n = 20)		Non-hospitalized (n = 20)		p-Value
	n	Percent	n	percent	
Chronic cough	18	90	18	90	0.999
Hemoptysis	4	20	8	40	0.168
Dyspnea	19	95	16	80	0.342
Fine crackles	1	5	5	25	0.064
Wheezing	4	20	5	25	0.282
Coarse crackles	0	0	2	10	0.947

Clinical findings were not significantly different in the two groups at the time of study (twenty years after exposure). Chi square with exact Fisher test.

Table 2
Comparison of pulmonary function tests in the hospitalized and non-hospitalized subjects exposed to sulfur mustard in 1987 Sardasht, Iran.

	Total (N = 40)		Non-hospitalized (N = 20)		Hospitalized (N = 20)		p-Value
	Mean	SD	Mean	SD	Mean	SD	
FVC %	79.5	17.9	86.3	16.2	72.4	17.1	**0.016**
FEV1 %	69.0	24.4	79.8	20.6	58.2	23.5	**0.006**
FEV1/FVC %	83.0	18.7	89.2	16.7	78.1	19.4	0.144
MMEF %	49.8	29.5	60.2	31.6	39.5	24.4	0.100
PEF %	65.1	19.5	72.0	18.8	56.8	17.5	**0.024**

Spirometry parameters FEV1, FVC and PEF are significantly lower in the hospitalized group indicating worse pulmonary condition.
FVC: Forced Vital Capacity; FEV1: Forced Expiratory Volume in 1 second.
MMEF: Maximum Mid Expiratory Flow; PEF: Peak Expiratory Flow, p = p-value, T-test.
All concentrations in pg/dl.
Bold data shows significant differences with p value < 0.05.

2.6. ELISA measurements

Human PMN elastase, IL-8/CXCL8, RANTES, MCP-1, fractalkine, and MMP-9 DuoSet® ELISA Development Kits (R&D Systems) were used to measure cytokine levels in the sera and sputum. This assay employs the quantitative sandwich enzyme immunoassay technique. The ELISA reader and washer were Stat-Fax 2100 and Stat-Fax 2600 (USA) respectively.

2.7. Statistical analysis

Statistical comparison among groups was performed using the Mann–Whitney or T-test according to the data types. Correlation between inflammatory mediators and pulmonary function parameters was computed using Spearman's rank correlation coefficient. Differences were considered statistically significant when $p \leq 0.05$. Data are presented as Mean (SD) and Median (first and third quartiles). Analyses of all the data were performed using SPSS software version 16.0 (Chicago, Illinois, USA).

3. Results

3.1. Clinical findings

Pulmonary clinical findings of the groups were not significantly different at the time of the study (Table 1) but spirometry findings were different and FEV1 (Forced Expiratory Volume in 1 second), FVC (Forced Vital Capacity), and PEF (Peak Expiratory Flow) were significantly lower ($p = 0.0006$, $p = 0.016$, $p = 0.024$ respectively) in the hospitalized group (Table 2).

3.2. Inflammatory mediators in sputum 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. Sputum and serum levels of IL-8, fractalkine, RANTES, MCP1, MMP-9, and PMN-elastase from forty SM-exposed males were measured and compared between hospitalized with non-hospitalized groups.

Sputum levels of fractalkine were significantly lower in the hospitalized (Median = 1.05; IQR = 0.41–2.62) group compared to the non-hospitalized (Median = 4.03; IQR = 0.94–8.20) group (Mann–Whitney test, $p = 0.042$, Table 3). However, serum levels of fractalkine were higher in the hospitalized (Mean ± SD = 2.08 ± 5.09) group than in the non-hospitalized (Mean ± SD = 0.53 ± 0.87) group (T-test, $p = 0.03$). Serum levels of PMN-elastase were also higher in the hospitalized group (Mean ± SD; 64,794.43 ± 26,820.08) than in the non-hospitalized group (Mean ± SD = 44,049.33 ± 17,675.85) ($p = 0.017$). There was no significant difference in other serum chemokine levels between the two study groups (Table 4).

3.3. Association of clinical findings and the inflammatory mediators

An association between clinical symptoms and sputum and serum levels of inflammatory mediators was demonstrated using a statistical evaluation with a Mann–Whitney.

MMP-9 was significantly lower in the sputum of patients suffering hemoptysis (patients with hemoptysis-Median (Q1–Q3) = 2161 (281.5–2396.0) versus patients without hemoptysis-Median (Q1–Q3) = 2490 (2411–2574.5)) ($p = 0.028$). Serum level of IL-8 in patients with rhonchi (Median = 2.946, IQR = 0–5.891, $p = 0.034$) and serum level of RANTES

Table 3
Sputum levels of IL-8, fractalkine, MMP-9, RANTES/CCL5, MCP1/CCL2, MCP1/CCL2, and PMN elastase in the hospitalized and non-hospitalized subjects exposed to sulfur mustard in 1987 Sardasht, Iran.

	Study group	N	Mean	SD	Median	Q1	Q3	p-Value
IL-8	Non-hospitalized	20	730.91	358.10	686.05	503.50	828.90	0.807
	Hospitalized	19	788.24	466.99	757.70	421.80	1085.00	
Fractalkine	Non-hospitalized	18	11.315	19.782	4.031	0.947	8.203	**0.042**
	Hospitalized	16	2.908	4.810	1.050	0.410	2.629	
MMP-9	Non-hospitalized	17	2019.4	888.5	2451.0	2040.0	2493.0	0.658
	Hospitalized	17	2304.0	595.6	2415.0	2291.0	2575.0	
RANTES/CCL5	Non-hospitalized	8	76.75	83.05	19.52	16.86	157.80	0.673
	Hospitalized	9	88.03	70.61	54.76	18.68	154.20	
MCP1/CCL2	Non-hospitalized	8	334.36	138.80	348.55	213.25	464.45	0.397
	Hospitalized	9	258.27	215.18	278.00	40.12	407.70	
PMN elastase	Non-hospitalized	16	8234.25	10,273.22	3920.50	1389.50	11,767.68	0.732
	Hospitalized	12	24,745.59	53,544.36	3817.00	1961.70	12,233.50	

The sputum specimen was separated and diluted with fourfold volume of PBS. Inflammatory mediators were measured using ELISA (R&D) technique.
Sputum fractalkine is significantly lower in the hospitalized group. No significant difference was seen in other factors. Some samples were missed for limited amount of samples and technical difficulties. Mann–Whitney test comparison between hospitalized and non-hospitalized; IL-8: interleukin 8; MMP-9: matrix metalloproteinases-9; RANTES: Regulated upon Activation, Normal T-cell Expressed, and Secreted; MCP1: monocyte chemoattractant protein-1; SD: standard deviation; Q1 = first quartile; Q3: third quartile. All concentrations in pg/dl.
Bold data shows significant differences with p value < 0.05.

Table 4
Serum levels of IL-8, fractalkine, MMP-9, RANTES, MCP1, MCP1, and PMN elastase in the hospitalized and non-hospitalized subjects exposed to sulfur mustard in 1987 Sardasht, Iran.

	Study group	N	Mean	SD	Median	Q1	Q3	p-Value
IL-8	Non-hospitalized	20	16.727	23.534	9.659	7.135	12.458	0.187
	Hospitalized	19	27.737	39.149	13.705	8.023	26.942	
Fractalkine	Non-hospitalized	18	0.53	0.87	0.29	0.17	0.48	**0.030**
	Hospitalized	16	2.08	5.09	0.57	0.38	1.01	
MMP-9	Non-hospitalized	17	949	719	741	443	943	0.150
	Hospitalized	17	2540	3337	1014	628	2503	
RANTES/CCL5	Non-hospitalized	8	833.41	383.29	794.00	570.80	982.60	0.963
	Hospitalized	9	909.34	704.18	730.60	516.40	1025.00	
MCP1/CCL2	Non-hospitalized	8	198.545	79.130	178.500	155.550	194.150	0.815
	Hospitalized	9	211.871	106.392	187.300	154.600	259.600	
PMN elastase	Non-hospitalized	16	44,049.33	17,675.85	41,086.94	34,670.87	51,142.08	**0.017**
	Hospitalized	12	64,794.43	26,820.08	56,594.82	45,680.22	77,881.31	

Inflammatory mediators were measured using ELISA (R&D) technique.
Serum fractalkine and PMN-elastase are significantly higher in the hospitalized group.
Mann–Whitney test comparison between hospitalized and non-hospitalized; IL-8: interleukin 8; MMP-9: matrix metalloproteinases-9; RANTES: Regulated upon Activation, Normal T-cell Expressed, and Secreted; MCP1: monocyte chemoattractant protein-1; SD: standard deviation; Q1: first quartile; Q3: third quartile. All concentrations in pg/dl.
Bold data shows significant differences with p value<0.05.

in patients with hemoptysis (Median = 473.35, IQR = 409–537.60, p = 0.040) were significantly lower than those without such symptoms. There was no association between the other studied parameters and the remaining pulmonary symptoms and signs.

There was no relation between any measured factors in the sputum and the GOLD classification. However, serum levels of fractalkine (Median = 0.598, IQR = 0.462–1.060, p = 0.28) and MMP-9 (Median = 1692.5, IQR = 914.3–4292, p = 0.011) were significantly higher in more severe group (grades 3 and 4 GOLD classification). In addition, serum PMN-elastase level was only in the moderate group (grade 2 GOLD classification) significantly higher (Median = 71,626.3, IQR = 55,504.8–108,911.5, p = 0.040) than in patients with normal spirometry.

3.4. Correlations

There was a negative correlation between sputum fractalkine level and serum levels of MMP-9 and PMN-elastase and there was a positive correlation between sputum PMN-elastase level and serum MCP1 level. No correlation exists between the other studied parameters in the sputum and serum (Table 5).

The correlations between sputum chemokines were shown in Table 6. Sputum MCP-1 levels have a positive correlation with sputum fractalkine and a negative correlation with sputum RANTES levels. Sputum fractalkine levels also had a significantly direct and reverse correlation with MN and PMN sputum cell percentages, respectively.

There was no correlation between sputum chemokine, MMP-9, and PMN-elastase and spirometry parameters. However, FEV1 showed a significant inverse correlation with the sputum PMN count and a significant direct correlation with sputum MN count (Table 7).

4. Discussion

In this study the association of sputum and serum chemokines, MMP-9, PMN-elastase and sputum cell distribution with severity of pulmonary involvement in SM exposed subjects was evaluated. The sputum and serum levels of the above factors showed no significant difference between the hospitalized and non-hospitalized groups which seemingly suggested no association with the severity of symptoms at the time of exposure twenty years earlier.

It has been reported that IL-8/CXCL8 has a significant role in chronic pulmonary diseases like COPD and asthma and might serve as a biomarker for diagnosis and disease activity. Furthermore, increased levels of IL-8/CXCL8 in the sputum and BALF have been reported in patients with COPD, asthma, bronchiolitis obliterans, and pulmonary fibrosis especially in exacerbation of diseases [8]. Emad and Emad reported the increased levels of IL-8/CXCL8 in the BALF of mustard gas exposed patients with pulmonary fibrosis [17]. Previously we have reported that the serum level of IL-8 was decreased in the SM exposed patients 20 years after exposure and there was no correlation between the serum level of IL-8 and pulmonary involvement [18]. The present study showed no significant difference between the sputum IL-8/CXCL8 level of the two SM exposed of hospitalized vs. non-hospitalized and no correlation was seen between IL-8 level and pulmonary symptoms, signs, and function tests. The differences between Emad and Emad's findings

Table 5
Correlations between serum and sputum levels of IL-8, fractalkine, MMP-9, RANTES, MCP1, MCP1, PMN elastase, and cell counts in 40 sulfur mustard exposed (1987), Sardasht, Iran.

		Serum					
		IL-8/CXCL8	Fractalkine/CX3CL1	MMP-9	RANTES/CCL5	MCP1/CCL2	PMN elastase
Sputum	IL-8/CXCL8	−0.069	0.131	0.271	−0.059	−0.135	0.075
	Fractalkine/CX3CL1	−0.234	−0.071	**−0.462***	−0.117	−0.201	**−0.438***
	MMP-9	0.026	0.028	0.113	0.060	0.011	0.053
	RANTES/CCL5	0.199	0.217	0.047	0.130	−0.190	0.098
	MCP-1/CCL2	−0.348	−0.075	−0.338	−0.051	0.170	−0.315
	PMN elastase	0.084	0.159	0.125	−0.238	**0.503***	−0.112
	PMN (percent)	0.176	−0.177	0.288	−0.097	0.325	−0.039
	MN (percent)	−0.178	0.177	−0.286	0.098	−0.321	0.039

Sputum fractalkine shows a negative correlation with serum MMP-9 (matrix metalloproteinases-9) and PMN-elastase. Sputum PMN-elastase shows a positive correlation with serum MCP-1 (monocyte chemoattractant protein-1).
PMN: polymorphonuclear cells; MN: mononuclear cells; IL-8: interleukin 8; RANTES: Regulated upon Activation, Normal T-cell Expressed, and Secreted; MCP1: monocyte chemoattractant protein-1. All concentrations in pg/dl.
Bold data shows significant differences with p value<0.05.
* p-Value<0.05. Spearman's correlations (significant correlation presented with asterisk).

Table 6
Correlations between sputum levels of IL-8, fractalkine, MMP-9, RANTES, MCP1, MCP1, PMN elastase, and cell counts in 40 sulfur mustard exposed (1987), Sardasht, Iran.

		Sputum							
		IL-8/CXCL8	Fractalkine/CX3CL1	MMP-9	RANTES	MCP-1	PMN elastase	PMN	MN
Sputum	IL-8/CXCL8		−0.043	0.193	0.074	−0.157	−0.326	−0.116	0.116
	Fractalkine			−0.206	−0.111	**0.561***	−0.098	**−0.409***	**0.409***
	MMP-9				0.119	−0.439	0.139	0.242	−0.242
	RANTES					**−0.520***	−0.321	−0.164	0.164
	MCP-1						0.421	−0.100	0.100
	PMN elastase							0.399	−0.391

Sputum fractalkine shows a positive correlation with sputum MCP-1 and monocyte cell (MN) count but a negative correlation with sputum polymorphonuclear cell (PMN) count. Negative correlation exists between sputum RANTES (Regulated upon Activation, Normal T-cell Expressed, and Secreted) and sputum MCP-1 (monocyte chemoattractant protein-1); IL-8: interleukin 8; MMP-9: matrix metalloproteinases-9. All concentrations in pg/dl.
Bold data shows significant differences with p value<0.05.
* p-Value<0.05. Spearman's rho correlations (significant correlation presented with asterisk).

(2007) and the current study might be due to the differences in design, methodology, and especially different population. They studied the BALF of patients with pulmonary fibrosis, comparing SM exposed subjects with healthy and unexposed subjects. However, the present study compared the sputum measurements of a subgroup of SICS subjects that was selected by randomized sample selection.

There is a report which showed that pulmonary function is negatively correlated with sputum IL-8 in subjects with cystic fibrosis but not in those with chronic bronchitis [19]. PMN elastase which had increased in the serum of hospitalized group might be responsible for the increased pathologic lung changes and worsening pulmonary function in this group. However, there was no correlation between this enzyme and spirometry parameters. Interestingly, bronchiolitis obliterans (BO) was related to the BALF levels of PMN elastase [20,21]. Ghanei et al. reported BO as the main lung pathology of subclinical exposure to SM [22]. Unexpectedly, sputum level of PMN elastase was not different in the two groups. The reason may be that PMN elastase markedly increases during infections and in the study the patients with acute febrile infections were excluded.

Growing amounts of research have been devoted to investigate the critical role of increased fractalkine/CX3CL1 levels in chronic inflammatory lung diseases such as COPD and pulmonary hypertension [11]. In the previous study done by the same group, serum fractalkine was not different between 372 SM exposed individuals and the control group [6]. The present study showed decreased sputum level and interestingly increased serum levels of fractalkine/CX3CL1 in the hospitalized group. One likely explanation might be the destruction of other factors such as PMN elastase. Alternatively, a different pathophysiologic process possibly involved systemic responses in SM lung complications. The data showed no correlation between the sputum and serum levels of CX3CL1 and severity of lung function impairment as measured by spirometry. To the best of our knowledge, there has been no prior investigation of the sputum or BALF fractalkine/CX3CL1 of SM exposed victims.

We measured sputum and serum MMP-9 levels but not MMP-9 activity which might be a limitation in the analysis of the current study. It is likely that the MMP-9 activation influences the alveolar level, since the enzyme is rapidly inactivated and so measurement of sputum levels of this enzyme might not be accurate.

The results of this study suggest no chemokine-induced inflammatory process exists in the sputum and serum of patients 20 years after exposure to SM. However, one might discuss that the sample size was not enough but there is no choice to include more. The investigations with a larger sample size are suggested.

Significantly lower FEV1, FVC, and especially PEF in the hospitalized group suggested more impairment of pulmonary function in the hospitalized group. Nevertheless, the data showed no significant difference in the majority of chemokines between the two groups and therefore no correlation between the severity of pulmonary involvement and sputum levels of measured chemokines and enzymes in this group of SM exposed patients was seen.

It has been reported that neutrophils and eosinophils accumulate in the lung secretions of COPD patients [23] and a study of SM exposed populations indicated the eosinophilic pattern of BALF [17]. However, we found an inverse correlation between sputum PMN count and pulmonary function tests and a direct correlation between MN count and pulmonary function tests.

In conclusion the results showed no significant correlation between sputum and serum levels of inflammatory mediators and long term pulmonary complications twenty years after exposure to SM and also with the severity of pulmonary involvement at the time of exposure. The data suggest that in comparison with chronic pulmonary disease

Table 7
Correlations between sputum levels of IL-8, fractalkine, MMP-9, RANTES, MCP1, MCP1, PMN elastase, cell counts and spirometry parameters in 40 sulfur mustard exposed (1987), Sardasht, Iran.

		Spirometry parameters				
		FVC %	FEV1 %	FEV1/FVC %	MMEF %	PEF %
Sputum	IL-8/CXCL8	−0.043	−0.089	0.212	0.095	−0.085
	Fractalkine/CX3CL1	0.252	0.238	0.304	0.247	−0.024
	MMP-9	−0.059	−0.033	0.047	−0.042	−0.204
	RANTES/CCL5	0.154	−0.014	−0.167	−0.500	−0.159
	MCP-1/CCL2	0.139	0.100	0.071	−0.179	0.121
	PMN elastase	−0.069	−0.239	−0.416	−0.140	−0.145
	PMN (percent)	−0.399	**−0.425***	−0.138	−0.061	−0.186
	MN (percent)	0.399	**0.425***	0.138	0.061	0.186

Only FEV1 shows a negative correlation with sputum polymorphonuclear cell (PMN) count and positive with sputum monocyte cell (MN) count. IL-8: interleukin 8; MMP-9: matrix metalloproteinases-9; RANTES: Regulated upon Activation, Normal T-cell Expressed, and Secreted; MCP1: monocyte chemoattractant protein-1. All concentrations in pg/dl.
Bold data shows significant differences with p value<0.05.
* p-Value<0.05. Spearman's rho correlations (significant correlation presented with asterisk).

(COPD) other pathophysiologic and regulatory processes may be involved. Considering the important role of chemokines in lung inflammation and lung diseases, it seems that additional research could clarify the picture. However, further insight into the role of inflammatory mediators in the pathogenesis of SM requires additional investigation of the molecular and cellular mechanisms involved. This information is needed to provide a more accurate evaluation and therapeutic strategy when dealing with victims of SM exposure.

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