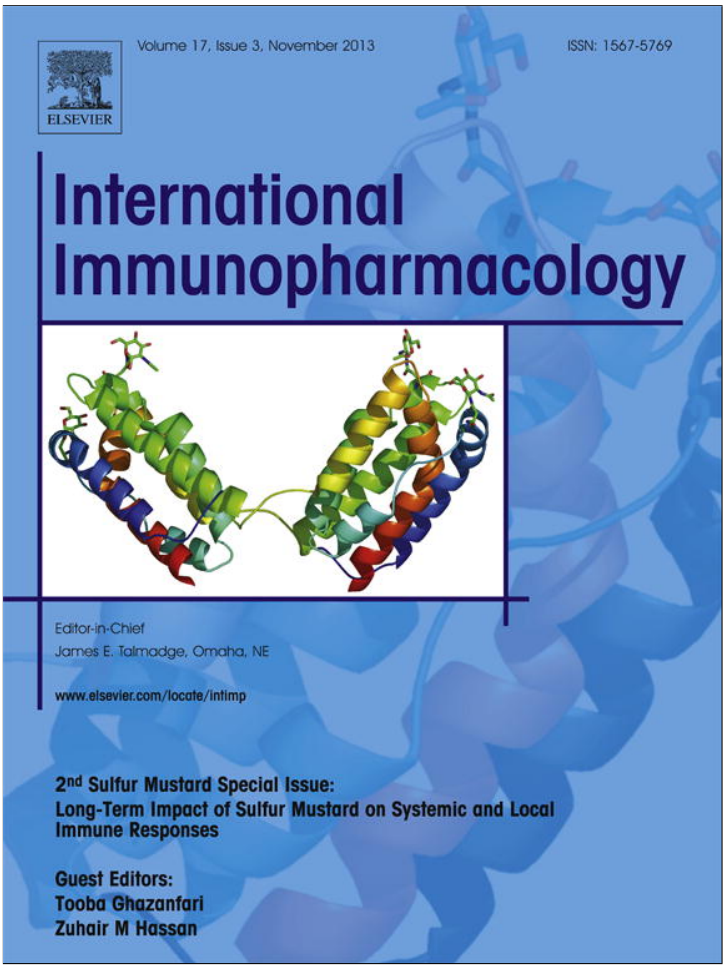

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Are serum levels of immunoglobulin classes and IgG subclasses involved in delayed pulmonary complications induced by sulfur mustard? Sardasht-Iran Cohort Study

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ABSTRACT

Respiratory complications are the foremost long term debilitating effects after sulfur mustard toxicity. The underlying immunological mechanisms of sulfur mustard induced lung damage are still poorly understood. The question of the involvement of immunoglobulin classes and subclasses in delayed pulmonary complications induced by SM was addressed in this study as a part of Sardasht-Iran Cohort Study (SICS).In SICS, 372 male participants who were exposed to SM 20 years earlier were compared with 128 unexposed age-matched controls. At the time of study (2007), the clinical evaluations and spirometry was performed for all subjects according to the American Thoracic Society Criteria, and at the same time, the sera were isolated, labeled and aliquots were kept frozen in −80 °C. Serum immunoglobulin (Ig) levels including IgM, IgA, IgE, IgG, and IgG subclasses (IgG1, IgG2, IgG3 and IgG4) were measured using quantitative Elisa method. It was found that among immunoglobulin classes and IgG subclasses only IgM and IgG4 were significantly decreased in the peripheral blood of exposed cases. IgM level also positively correlated with FEV1 only in the SM exposed group. These results indicated a weak but significant role for IgA in control of the delayed pulmonary complications. There were no strong correlations between other immunoglobulin classes or IgG subclasses with pulmonary disease severity in sulfur mustard intoxicated subjects. The authors proposed that systemic levels of immunoglobulins do not exert essential roles in severity of delayed pulmonary complications following SM toxicity. However, more studies on local and systemic levels of immunoglobulins in more severe groups are suggested.

1. Introduction

Sulfur mustard (SM) or mustard gas induces short term toxicity and long term toxicity in different organs including the eyes, skin, lungs and the hematopoietic system. The most clinically serious long-term consequences of SM toxicity are to the respiratory system. It was reported that almost 95% of SM exposed individuals have at least one respiratory sign or several symptoms [1,2]. It has been reported that SM exposure can lead to the development of a series of chronic destructive pulmonary complications. Most of the intoxicated populations have numerous respiratory symptoms such as cough, sputum, hemoptysis, and chest pain. Respiratory complications exacerbate over the time. Cough was found to be the most common symptom affecting almost all of the exposed subjects [3]; however, some studies found the shortness of breath to be the most common symptom [4]. Asthma, chronic bronchitis, bronchiectasis, pulmonary fibrosis [5], oblitrative bronchiolitis (OB) and chronic obstructive pulmonary disease (COPD) [6,7] are the main respiratory disorders reported.

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Immunoglobulins play several important roles in pathogenesis of inflammatory pulmonary diseases such as asthma, emphysema, chronic bronchitis, BO and IPF [8–10]. They have also been considered as biomarkers of pulmonary disease severity or exacerbations [11]. Increased level of some immunoglobulins was reported in bronchoalveolar lavage fluid in SM exposed patients [12]. The immunological mechanisms underlying sulfur mustard induced lung injuries are still poorly understood.

To understand the implications of SM-exposed chemical injuries, a comprehensive historical cohort study, Sardasht-Iran Cohort Study (SICS) was performed. The study used a civilian population of Sardasht town who were exposed to SM 20 years before in comparison with a corresponding unexposed control group [13]. SICS investigated the molecular patterns of systemic and local immune responses, inflammation, hematologic and biochemical parameters along with clinical, lifestyle, and psychological factors. Clinical findings of SICS are in õconcordance with previous reports which found that respiratory, eye and skin complications were the main problems experienced by the SM exposed population [3,14,15]. Hematological results showed a significant reduction in WBC and an elevation in lymphocytes in this population in comparison to the unexposed control group [16]. Our previous reports also revealed a significant reduction in systemic pro-inflammatory cytokines and chemokines in the majority of cases without any relation to pulmonary problems [17,18].

In the present study, we addressed the question whether immunoglobulin classes and IgG subclasses are involved in delayed pulmonary complications induced by SM in the context of SICS.

2. Materials and methods

2.1. Study design and participants

Details of the study design and methods of SICS were reported previously. Briefly, in SICS, 372 male participants from Sardasht who were exposed to SM in 1987 were compared with 128 unexposed age matched controls from the unexposed town of Rabat. SICS was started at 2006 and the clinical evaluations and serum and other sample preparations was undertaken at 2007, with the experiments being done during 6 months after sample preparation. The study was approved by the Ministry of Health of Iran, Janbazan Organization, Shahed University and Janbazan Ethic Committees. Informed consent was obtained from all the participants. Complete methodological details of SICS and demographic information were reported previously in the original methodology paper [13].

2.2. Serum preparation

Blood drawn into non-treated Vacutainer tubes (BD Biosciences) was used for serum preparation. After clotting, the sera were isolated and labeled and aliquots were kept frozen in −80 °C.

2.3. Clinical evaluation

A questionnaire of pulmonary symptoms (chronic cough, sputum, hemoptysis, and dyspnea) and pulmonary findings (crackles, rales, and wheezing) was completed by three internists who examined the patients at the same time [18]. Spirometry was performed on all the participants according to the American Thoracic Society Criteria with a spirometry device (Chest 801 Spirometry). The severity of pulmonary involvement was classified according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD). Classification and clinical assessment also used criteria verified by the Iranian Medical Committee of the Foundation of Martyr and Veterans Affairs [19]. There was no grade IV severity among admitted subjects according to GOLD classification and only subjects in grades I–III were used. Chronic cough was defined as cough for more than four weeks. Dyspenea severity was classified according to the MRC breathless scale [20].

2.4. Immunoglobulin measurement

A capture ELISA was used to determine the levels of immunoglobulin classes and IgG subclasses. Anti-IgG, -IgA, -IgM and -IgE (Bethyl Laboratories, Montgomery, USA) and anti-IgG1, -IgG2, -IgG3 and -IgG4 (Sigma, St. Louis, MO, USA) were coated (5 μg/ml) for 2 h at 37 °C. After two washings with phosphate buffered saline, pH 7.2 containing 0.05% (v/v) Tween 20 (PBS-T), nonspecific sites were blocked with PBS-T containing BSA 1% (w/v) for 1 h followed by three washes with PBS-T. 100 μl of serum samples (diluted 1:10,000 for IgG and IgA, 1:6000 for IgM, 1:5 for IgE, 1:1000 for IgG1, 1:500 for IgG2 and 1:100 for IgG3 and IgG4) was added to wells in duplicate and incubated for 1 h at RT. After washing five times with PBS-T, 100 μl of HRP-conjugated detecting antibodies specific for each immunoglobulin class (Bethyl Laboratories, Montgomery, USA) or subclass (Sigma, St. Louis, MO, USA) was added at appropriate dilutions. Plates were incubated for 1 h at RT and after washing five times, 100 μl of TMB substrate solution (Sigma, St. Louis, MO, USA) was added. After 20 min of incubation in a dark place, the reaction was stopped with 100 μl of 5% (v/v) solution of sulfuric acid and read by a plate reader (Awareness, USA) at 450 nm.

2.5. Statistical analysis

Data was presented as mean ± standard deviation (SD). Data analysis was performed by SPSS, version 16 (SPSS Inc, Chicago, USA), using ANOVA and Student *T*-test to compare the study groups. Differences were considered statistically significant when p was ≤ 0.05. Pearson's correlation was done to find correlations between immunoglobulin levels and spirometry parameters.

3. Results

3.1. Serum immunoglobulin levels in study groups

Fig. 1 shows a significant decrease in the serum levels of IgM in the exposed group compared to the control ($p = 0.002$). There were no significant differences between control and exposed group regarding serum level of IgG, IgA and IgE. The results of IgG subclasses were presented in Fig. 2, a significant decrease in IgG4 level was revealed in the serum of the exposed group compared to the control ($p = 0.04$). There were no significant differences between serum level of other IgG subclasses (IgG1, IgG2 and IgG3) in control and exposed groups.

3.2. Association of the serum levels of immunoglobulin classes and IgG subclasses with pulmonary signs and symptoms

Within the exposed group, patients with hemoptysis ($p = 0.041$) or severe dyspnea ($p = 0.004$) had a decreased serum level of IgM. Serum IgM was also decreased significantly in exposed people suffering chronic cough ($p = 0.003$), sputum ($p = 0.01$), and hemoptysis ($p = 0.025$) when compared with the controls(Table 1).

The findings revealed significant associations between chronic cough and the level of IgA (mean IgA = 3.886 mg/ml for who had chronic cough and 3.508 mg/ml for those who did not have chronic cough $p = 0.049$) only in the control group. The control group have also revealed significant negative associations between sputum and IgG (mean IgG = 16.45 mg/ml for who had sputum and mean = 19 mg/ml for who did not have sputum $p = 0.001$). However, there were no significant associations between serum levels of IgG, IgA, IgE, and IgG subclasses with pulmonary symptoms of chronic cough, sputum, hemoptysis, and dyspnea severity within exposed group and also between the exposed and control group(data were not shown).

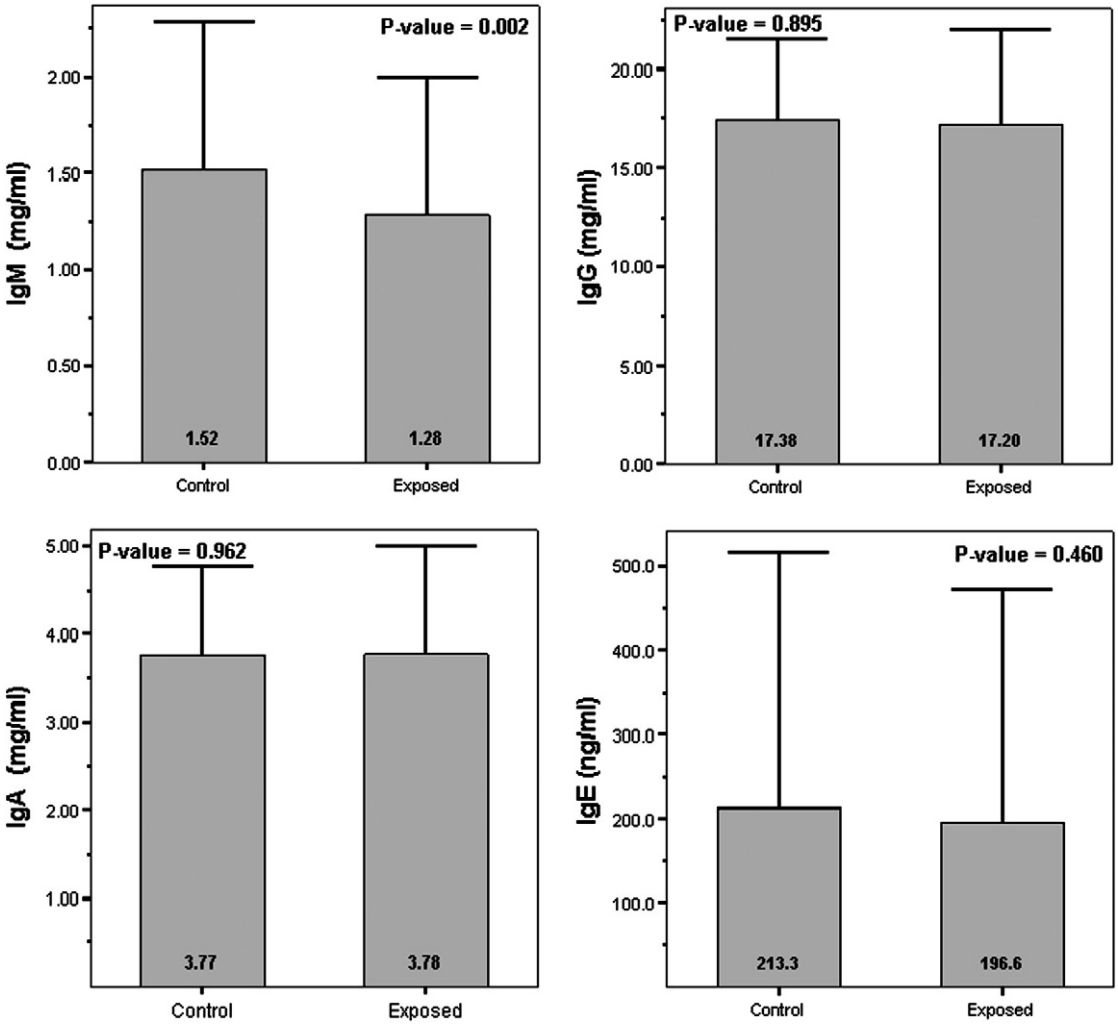

Fig. 1. Serum levels of immunoglobulin classes were assessed in all participants including the control and exposed groups by Elisa method. Data was presented as mean ± SD. p-Value: comparison of the exposed with the control group was undertaken by ANOVA (*T*-test).

Pulmonary findings of rales, rhonchi, and wheezing had no associations with serum immunoglobulin classes or IgG subclasses either within each study group or between control and exposed groups (data were not shown).

3.3. Comparison of the serum levels of immunoglobulin classes and IgG subclasses between control and exposed groups at different stages of pulmonary involvement

A comparison was made between spirometry parameters of the control and exposed groups. As shown in Table 2, all of the spirometry findings were significantly decreased in the exposed group as compared to the control group.

Table 3 shows the comparison of serum levels of immunoglobulin classes at different stages of pulmonary involvement according to the clinical assessment. As shown in Table 3, there were significant differences in the serum levels of IgM between control and parallel exposed groups with normal and mild pulmonary assessment. In addition, serum level of IgA shows statistically significant differences between groups with different stages of pulmonary involvement only in the exposed participants (0.008). There were no significant differences regarding the serum level of other immunoglobulin classes in different stages of severity of pulmonary involvement between the control and exposed groups.

Table 4 shows the comparison of serum levels of IgG subclasses at different stages of pulmonary involvement according to the clinical assessment. As shown in Table 4, there was significant increase in the serum levels of IgG1 in exposed groups with normal and moderate–severe pulmonary assessment compared to the parallel control groups. In addition, serum level of IgG1 shows statistically significant differences within control groups with different stages of pulmonary involvement. There was a significant decrease in the serum levels of IgG4 in the exposed group with normal pulmonary assessment when compared to the parallel control group. There were no significant differences regarding the other IgG subclasses in different stages of severity of pulmonary involvement between control and exposed groups.

3.4. Association of serum levels of immunoglobulin classes and IgG subclasses with pulmonary problem severity

There were no associations seen between the serum levels of immunoglobulin classes and pulmonary function according to GOLD (Global Initiative for Chronic Obstructive Lung Disease) classification within control or exposed groups (Table 5). In evaluations, only a significant decrease was observed in serum levels of IgM in exposed group who had normal pulmonary condition when compared to the parallel control group (Table 5).

In addition, there were no significant differences between serum levels of IgG subclasses (Table 5). However, a significant difference was observed between the serum levels of IgG3 in the control group with/without pulmonary problems (Table 6).

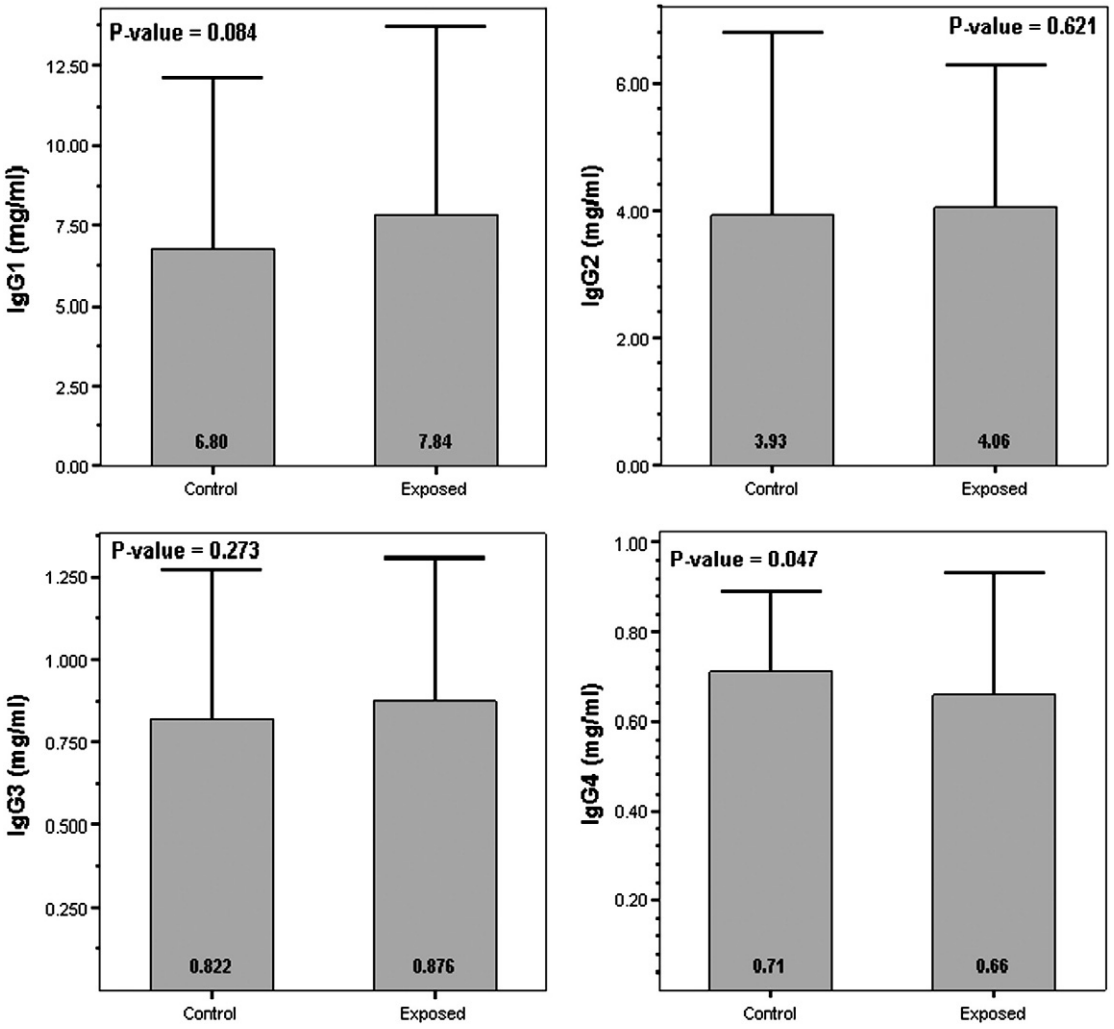

Fig. 2. Serum levels of four IgG subclasses were assessed in all participants including the control and exposed groups by Elisa method. Data was presented as mean ± SD. p-Value: comparison of the exposed with the control groups was undertaken by ANOVA (*T*-test).

Table 1
Association of the serum levels of IgM and pulmonary symptoms in the SM exposed and control group.

		IgM (mg/ml)								
		Control				Exposed				p-Value[2]
		N	Mean	SD	p-Value[1]	N	Mean	SD	p-Value[1]	
Chronic cough	Yes	79	1.534	0.713	0.917	327	1.274	0.701	0.526	**0.003**
	No	41	1.518	0.886		22	1.372	0.738		0.512
Cough pattern	Always	12	1.526	0.657	0.913	87	1.303	0.755	0.637	0.333
	Some time	56	1.554	0.781		189	1.239	0.706		**0.005**
	Little	8	1.436	0.410		49	1.328	0.559		0.603
Cough severity	Is not problem	49	1.666	0.750	0.094	110	1.272	0.619	0.526	**0.001**
	Sometimes problem	24	1.320	0.659		175	1.269	0.764		0.757
	Problematic	2	1.009	0.006		30	1.118	0.567		0.790
Sputum	Yes	77	1.521	0.769	0.888	314	1.287	0.699	0.622	**0.010**
	No	43	1.542	0.788		35	1.225	0.746		0.074
Hemoptesi	Yes	15	1.528	0.721	0.997	117	1.184	0.531	**0.041**	**0.025**
	No	105	1.529	0.783		232	1.329	0.772		**0.029**
Dyspnea severity	High activity	76	1.505	0.811	0.570	178	1.315	0.650	**0.004**	**0.049**
	Normal activity	11	1.536	0.701		60	1.378	0.943		0.599
	Low activity or in rest	6	1.155	0.683		82	1.044	0.503		0.610

The serum levels of immunoglobulin classes were assessed in the SM exposed and control groups by Elisa method. A comparison of the serum levels of immunoglobulin classes was undertaken between participants who had and who did not have any pulmonary symptoms (chronic cough, cough pattern, sputum, hemoptysis, and dyspnea severity). Data presented as mean ± SD. Bold data shows significant differences with p value <0.05.
p-Value[1]: Comparison of pulmonary symptom (Yes and No) groups (*T* test or ANOVA).
p-Value[2]: Comparison of study (Control and Exposed) groups (*T* test).

Table 2
Comparisons of the spirometry findings between study groups.

	Study groups		p value
	Control N = 128	Exposed N = 372	
FVC%	93.92 ± 17.04	86.68 ± 17.19	<0.001
FEV1%	89.14 ± 19.69	81.08 ± 19.73	<0.001
FEV1/FVC%	98.25 ± 11.03	94.61 ± 13.58	0.045
MMEF%	78.98 ± 34.08	67.12 ± 29.79	0.008
PEF%	83.16 ± 20.25	76.91 ± 20.33	0.004

Spirometry was performed on all the participants according to the American Thoracic Society Criteria with spirometry device (Chest 801 Spirometry). FVC, FEV1, MMEF and PEF are the parameters of pulmonary function test that were done by spirometry that are presented as percent and a comparison was made between control and expose groups. Differences were considered statistically significant when p was ≤0.05. Forced vital capacity, FEV1: forced expiratory volume in 1 s, MMEF: maximum midexpiratory flow, PEF: peak expiratory flow.

3.5. Correlation of the serum levels of immunoglobulin classes and IgG subclasses with spirometry parameters

Pearson correlation coefficient was undertaken to find out any correlations between serum levels of Ig classes and pulmonary function tests within each study (control and exposed) groups. There were low positive correlations but statistically significant between the serum levels of IgM and FEV1% ($p = 0.028$) only in the exposed group. Furthermore, a low but statistically significant correlation was found between the serum level of IgA and MMEF% ($p = 0.033$) only in exposed group. However, there were no significant correlations between the values of spirometry parameters and the serum levels of other immunoglobulin classes and IgG subclasses in the exposed group (Table 7).

4. Discussion

The role of systemic and local immunoglobulin levels in different chronic inflammatory lung diseases has been addressed in various studies. A regulatory role for immunoglobulin is reported on tissue forming cells relevant in chronic inflammatory lung diseases [21]. It is also proposed that IgG subclass deficiencies contribute to the development and progression of respiratory disease in COPD patients [10].

There were a few reports on immunoglobulin classes in SM exposed people; however, the role of systemic and local immunoglobulin levels in various local or systemic complications induced by SM is not clearly understood. This study, as a part of Sardasht-Iran Cohort Study, was designed to evaluate the probable role of immunoglobulin classes and IgG subclasses in delayed pulmonary complications induced by sulfur mustard.

The results showed a significant decrease in serum levels of IgM in the exposed group in comparison to the control group. There were no significant differences regarding the serum levels of the other immunoglobulin classes between study groups.

A significant decrease in serum levels of IgG in SM exposed patients on the third day after SM exposure has been reported previously [22]. However, an increased level of IgG was reported in the serum samples collected from the patients 4 to 18 days after exposure to SM. They proposed the possible leakage of IgG into severely affected parts of the body such as skin and respiratory system in acute phase, whereas they pointed out that the subsequent increase in serum IgG is interpreted due to (auto) antigenic stimulation of the patients' immune systems. The present study is undertaken on civilian inhabitants who were exposed to SM 20 years before study; therefore the aforementioned findings are not comparable to our results. In contrast to our results, Mahmoudi et al. illustrated a significant elevation only in IgM levels of sera in a group of veterans who had shown severe complications 16 to 20 years after SM exposure [23]. The different results could be due to differences in disease severity in different organs, the quantity and frequency of exposure to SM, and other parameters which must be considered. However, our results showed this reduction only in IgM levels in the exposed groups with normal or mild pulmonary assessment (according to clinical assessment using criteria verified by the Iranian Medical Committee of the Foundation of Martyr and Veterans Affairs) and those exposed without pulmonary problems (according to GOLD classification); however this was not consistent in the group with more severe lung problems. On the other hand, we found positive correlations between IgM levels and FEV1% only in the SM exposed group. Therefore, we proposed that although the level of IgM is

Table 3
Comparison of the serum levels of immunoglobulin classes in control and exposed groups with different stages of pulmonary problems based.

Immunoglobulin	Groups	Pulmonary assessment				p-Value[1]
			Normal	Mild	Moderate–severe	
IgM (mg/ml)	Control	N	84	29	7	0.274
		Mean ± SD	1.553 ± 0.752	1.570 ± 0.813	1.073 ± 0.817	
	Exposed	N	203	98	47	0.208
		Mean ± SD	1.332 ± 0.731	1.242 ± 0.656	1.143 ± 0.669	
	p-Value[2]		**0.022**	**0.027**	0.800	
IgG (mg/ml)	Control	N	84	29	7	0.426
		Mean ± SD	17.405 ± 4.642	17.717 ± 3.263	15.391 ± 2.077	
	Exposed	N	203	98	47	0.854
		Mean ± SD	17.297 ± 4.634	17.287 ± 5.043	16.864 ± 5.444	
	p-Value[2]		0.857	0.667	0.485	
IgA (mg/ml)	Control	N	84	29	7	0.995
		Mean ± SD	3.759 ± 0.902	3.744 ± 1.311	3.781 ± 0.778	
	Exposed	N	203	98	47	**0.008**
		Mean ± SD	3.935 ± 1.184	3.474 ± 1.157	3.713 ± 1.433	
	p-Value[2]		0.174	0.286	0.904	
IgE (ng/ml)	Control	N	84	29	7	0.163
		Mean ± SD	187.49 ± 283.49	301.87 ± 390.24	117.58 ± 89.68	
	Exposed	N	203	98	47	0.438
		Mean ± SD	189.15 ± 268.15	231.84 ± 300.64	188.61 ± 278.46	
	p-Value[2]		0.963	0.307	0.509	

The serum levels of IgM, IgG, IgA, and IgEin control and exposed groups at different severity stages of pulmonary assessment were presented as mean ± SD. Bold data shows significant differences with p value <0.05.
Pulmonary assessment: the classification of severity of pulmonary involvement in SM exposed patients according to clinical assessment using criteria verified by the Iranian Medical Committee of the Foundation of Martyr and Veterans Affairs.
p-Value[1]: Comparison of pulmonary groups, normal, mild and moderate–severe (*T* test or ANOVA).
p-Value[2]: Comparison of parallel study groups, control and exposed (*T* test).

Table 4
Comparison of the serum levels of IgG subclasses in control and exposed groups with different stages of pulmonary problems.

Immunoglobulin	Groups	Pulmonary assessment				p-Value[1]
			Normal	Mild	Moderate–severe	
IgG1 (mg/ml)	Control	N	84	29	7	**0.004**
		Mean ± SD	6.154 ± 4.639	9.770 ± 6.829	4.663 ± 2.077	
	Exposed	N	203	98	47	0.439
		Mean ± SD	7.704 ± 6.209	7.659 ± 5.769	8.890 ± 5.276	
	p-Value[2]		**0.040**	0.103	**0.001**	
IgG2 (mg/ml)	Control	N	84	29	7	0.461
		Mean ± SD	3.744 ± 2.630	4.516 ± 3.864	3.618 ± 1.036	
	Exposed	N	203	98	47	0.275
		Mean ± SD	3.906 ± 1.983	4.237 ± 2.388	4.392 ± 2.931	
	p-Value[2]		0.612	0.639	0.495	
IgG3 (mg/ml)	Control	N	74	21	5	0.359
		Mean ± SD	0.798 ± 0.489	0.837 ± 0.337	1.100 ± 0.390	
	Exposed	N	190	93	44	0.213
		Mean ± SD	0.853 ± 0.400	0.883 ± 0.405	0.976 ± 0.527	
	p-Value[2]		0.352	0.630	0.614	
IgG4 (mg/ml)	Control	N	84	29	7	0.495
		Mean ± SD	0.723 ± 0.180	0.702 ± 0.201	0.643 ± 0.056	
	Exposed	N	203	98	47	0.974
		Mean ± SD	0.662 ± 0.206	0.655 ± 0.405	0.655 ± 0.224	
	p-Value[2]		**0.019**	0.554	0.884	

The serum levels of IgG1, IgG2, IgG3, and IgG4 in control and exposed groups at different severity stages of pulmonary assessment were presented as mean ± SD. Bold data shows significant differences with p value <0.05.
Pulmonary assessment: the classification of severity of pulmonary involvement in SM exposed patients according to clinical assessment using criteria verified by the Iranian Medical Committee of the Foundation of Martyr and Veterans Affairs.
p-Value[1]: Comparison of pulmonary groups, normal, mild and moderate–severe (ANOVA, *T* test).
p-Value[2]: Comparison of parallel control and exposed groups (*T* test).

decreased on the whole of the exposed group, but it shows a positive correlation with pulmonary lung function tests, this means that within exposed group, IgM has a role not only in pathogenesis but also in the repair of pulmonary function. However the decrease in serum levels of IgM in normal and mild pulmonary assessments may be due to systemic suppression of immune system by SM, whereas the correlations of serum IgM are interpreted to be due to the patients' immune response to stimulation. It seems that the reduction in IgM in exposed group may be due to early onset of aging mechanisms in these patients, since it was reported that serum IgG and IgM concentrations are reduced in the elderly [24].

It should be noted that local evaluation of immunoglobulin classes is important for detailed understanding of the immunoglobulin roles in pulmonary complications induced by SM. It should also be noted that Emad and Rezaian reported higher levels of IgG in BAL fluid of SM patients with pulmonary fibrosis [12]. They did not found a

Table 5
Comparison of the serum levels of immunoglobulin classes in participant who had (with) and did not have (without) pulmonary problems.

Immunoglobulin	Groups		Pulmonary involvement based on GOLD classification		p-Value[1]
			Without problem	With problem	
IgM (mg/ml)	Control	N	109	12	0.437
		Mean ± SD	1.520 ± 0.746	1.341 ± 0.833	
	Exposed	N	292	57	0.138
		Mean ± SD	1.310 ± 0.730	1.157 ± 0.607	
	p-Value[2]		**0.011**	0.376	
IgG (mg/ml)	Control	N	109	12	0.269
		Mean ± SD	17.571 ± 4.313	16.148 ± 3.098	
	Exposed	N	292	57	0.065
		Mean ± SD	17.489 ± 5.003	16.201 ± 3.592	
	p-Value[2]		0.871	0.962	
IgA (mg/ml)	Control	N	109	12	0.818
		Mean ± SD	3.775 ± 1.026	3.705 ± 0.748	
	Exposed	N	292	57	0.582
		Mean ± SD	3.791 ± 1.216	3.693 ± 1.323	
	p-Value[2]		0.893	0.976	
IgE (ng/ml)	Control	N	109	12	0.799
		Mean ± SD	205.66 ± 309.47	182.46 ± 154.94	
	Exposed	N	292	57	0.143
		Mean ± SD	208.16 ± 284.33	149.41 ± 232.32	
	p-Value[2]		0.939	0.640	

The serum levels of IgM, IgG, IgA, and IgE in participants with and without pulmonary problems were assessed and a comparison was undertaken between the control and exposed groups, as well as, within each group based on the extent of pulmonary impairment. Those patients with pulmonary problems included participants who had mild, moderate or severe pulmonary problems. Those without pulmonary problems included participants who had a normal situation based on GOLD classification. Bold data shows significant p value.
p-Value[1]: comparison of participants with and without pulmonary problems within each group (ANOVA, *T* test). p-Value[2]: comparison of exposed with control groups (*T*-test).
Ig: Immunoglobulin.
GOLD: Global Initiative for Chronic Obstructive Lung Disease.

Table 6
Comparison of the serum levels of IgG subclasses in participant who had (with) and did not have (without) pulmonary problems.

Immunoglobulin	Groups		Pulmonary involvement based on GOLD classification		p-Value[1]
			Without problem	With problem	
IgG1 (mg/ml)	Control	N	109	12	0.365
		Mean ± SD	6.651 ± 5.046	8.759 ± 7.575	
	Exposed	N	292	57	0.969
		Mean ± SD	7.797 ± 6.120	7.763 ± 5.166	
	p-Value[2]		0.083	0.671	
IgG2 (mg/ml)	Control	N	109	12	0.360
		Mean ± SD	3.864 ± 2.874	4.675 ± 3.167	
	Exposed	N	292	57	0.259
		Mean ± SD	3.997 ± 2.120	4.363 ± 2.747	
	p-Value[2]		0.659	0.729	
IgG3 (mg/ml)	Control	N	92	10	0.058
		Mean ± SD	0.796 ± 0.451	1.082 ± 0.428	
	Exposed	N	276	53	0.588
		Mean ± SD	0.864 ± 0.404	0.903 ± 0.501	
	p-Value[2]		0.175	0.295	
IgG4 (mg/ml)	Control	N	109	12	0.543
		Mean ± SD	0.710 ± 0.177	0.743 ± 0.208	
	Exposed	N	292	57	0.288
		Mean ± SD	0.663 ± 0.287	0.621 ± 0.207	
	p-Value[2]		0.116	0.067	

The serum levels of IgG1, IgG2, IgG3, and IgG4 in participants with and without pulmonary problems were assessed and a comparison was undertaken between the control and exposed groups, as well as, within each group based on the extent of pulmonary impairment. Those patients with pulmonary problems included participants who had mild, moderate or severe pulmonary problems. Those without pulmonary problems included participants who had a normal situation based on GOLD classification.
p-Value[1]: comparison of participants with and without pulmonary problems within each group (ANOVA, *T*-test). p-Value[2]: comparison of exposed with control groups (*T*-test).
Ig: Immunoglobulin.
GOLD: Global Initiative for Chronic Obstructive Lung Disease.

correlation between IgM and severity of SM induced pulmonary disease. The elevated local levels of IgG in their study is parallel to Mahmoudi et al. study in which a systemic reduction in IgG was seen [23], because this reduction may be due to the leakage of IgG into involved organs. However, the present study did not show any alteration in systemic levels of IgG, which may be due to the lower severity of pulmonary problems in our study group. It is necessary to study the local and systemic levels of immunoglobulin in SM exposed patients with more severe pulmonary complications.

The results of the present study show that serum level of IgA in exposed group with normal pulmonary assessment is significantly more than those with mild and moderate–severe pulmonary assessment. Also a positive correlation was found between IgA and MMEF% only in exposed group. These results indicated a weak but significant role for IgA in control of the delayed pulmonary complications.

Various diagnoses have been reported for SM induced pulmonary complications, phatophysiological representation similar to, but in some aspects different from, OB and COPD were reported by Ghanei [6]. A correlation between IgE serum levels and clinical aspects of disease severity in COPD was reported previously [11]. A regulatory role for immunoglobulin is also reported on tissue forming cells relevant in chronic inflammatory lung diseases such as asthma and COPD, and this mechanism was presented as a reason for the reduced response of tissue forming cells after anti-IgE antibody therapy [21]. These differences in immunoglobulin levels in our study or other studies on SM exposed with COPD patients may

Table 7
Correlation of the serum levels of immunoglobulin classes and IgG subclasses with spirometry parameters in the control (N = 120) and SM exposed (N = 349) groups.

		FVC%		FEV1%		FEV1/FVC%		MMEF%		PEF%	
		Control	Expose	Control	Expose	Control	Expose	Control	Expose	Control	Expose
IgM	r	0.024	0.104	0.057	**0.118**	0.033	0.092	0.145	0.146	−0.012	0.010
	p	0.797	0.051	0.536	**0.028**	0.795	0.171	0.254	0.053	0.896	0.864
IgA	r	−0.144	0.067	−0.023	0.089	−0.003	0.029	0.013	**0.161**	0.026	0.035
	p	0.116	0.213	0.798	0.098	0.981	0.673	0.918	**0.033**	0.777	0.547
IgE	r	0.004	−0.031	−0.053	−0.001	−0.081	0.068	−0.010	−0.010	−0.070	0.056
	p	0.963	0.567	0.563	0.985	0.531	0.314	0.940	0.898	0.452	0.335
IgG	r	−0.075	−0.031	0.006	0.024	0.215	0.080	0.100	0.009	0.017	0.019
	p	0.413	0.569	0.949	0.655	0.088	0.235	0.432	0.902	0.851	0.741
IgG1	r	−0.030	−0.050	−0.052	−0.019	0.167	0.029	0.191	−0.016	0.049	0.022
	p	0.747	0.350	0.575	0.718	0.190	0.666	0.135	0.838	0.594	0.708
IgG2	r	0.029	−0.073	0.044	−0.066	−0.096	0.047	0.081	−0.025	0.142	0.012
	p	0.751	0.175	0.634	0.216	0.454	0.492	0.527	0.744	0.125	0.837
IgG3	r	−0.045	−0.063	−0.094	−0.071	−0.100	−0.116	−0.144	−0.082	−0.114	0.034
	p	0.656	0.255	0.349	0.200	0.463	0.097	0.288	0.297	0.255	0.568
IgG4	r	0.159	−0.023	0.092	−0.012	0.108	−0.033	0.176	−0.105	0.006	−0.023
	p	0.085	0.663	0.318	0.823	0.401	0.624	0.171	0.169	0.947	0.690

The serum levels of the immunoglobulin classes and IgG subclasses, and the pulmonary function parameters (FVC, FEV1, FEV1/FVC, MMEF, and PEF) were assessed. The correlation between the immunoglobulin classes and IgG subclasses and pulmonary function parameters was undertaken in the control and SM exposed groups. Bold data shows significant differences with p value <0.05. r: Spearman's correlation coefficient, p: p-value, FVC: forced vital capacity, FEV1: forced expiratory volume in 1 s, MMEF: maximum midexpiratory flow, PEF: peak expiratory flow.

indicate different phathophysiological mechanisms underlying SM induced pulmonary complications and those in COPD.

With regard to IgG subclasses, our results show a significantly decreased serum level of IgG4 of exposed group compared to the control, however when the study groups categorized based on their disease severity, this reduction was only found significant in the exposed group with normal pulmonary assessment when compared to the parallel control group. There was also a significant elevation in the serum levels of IgG1 in the exposed groups with normal and moderate–severe pulmonary assessment (based on criteria verified by the Iranian Medical Committee of the Foundation of Martyr and Veterans Affair) as compared to the parallel control groups. In addition, serum level of IgG1 shows statistically significant differences within control groups with different stages of pulmonary involvement. As far as we know, this is the first report on IgG subclasses in SM afflicted people. Regarding the results of present study, although some differences were found in serum levels of IgG1 and IgG4 subclasses in some exposed groups, these changes were not strongly correlated with SM induced pulmonary complications.

In conclusion, it was found that among immunoglobulin classes and IgG subclasses only IgM and IgG4 were significantly decreased in the peripheral blood of exposed cases. IgM level also positively correlated with FEV1 and IgA negatively associated with severity of pulmonary complications, only in the SM exposed group. There were no strong correlations between other immunoglobulin classes or IgG subclasses with pulmonary disease severity in sulfur mustard intoxicated subjects. We proposed systemic levels of immunoglobulin classes and IgG subclasses do not exert fundamental roles in severity of delayed pulmonary complications following SM toxicity. However, more studies at the local and systemic levels of immunoglobulins in more severe groups are suggested.

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