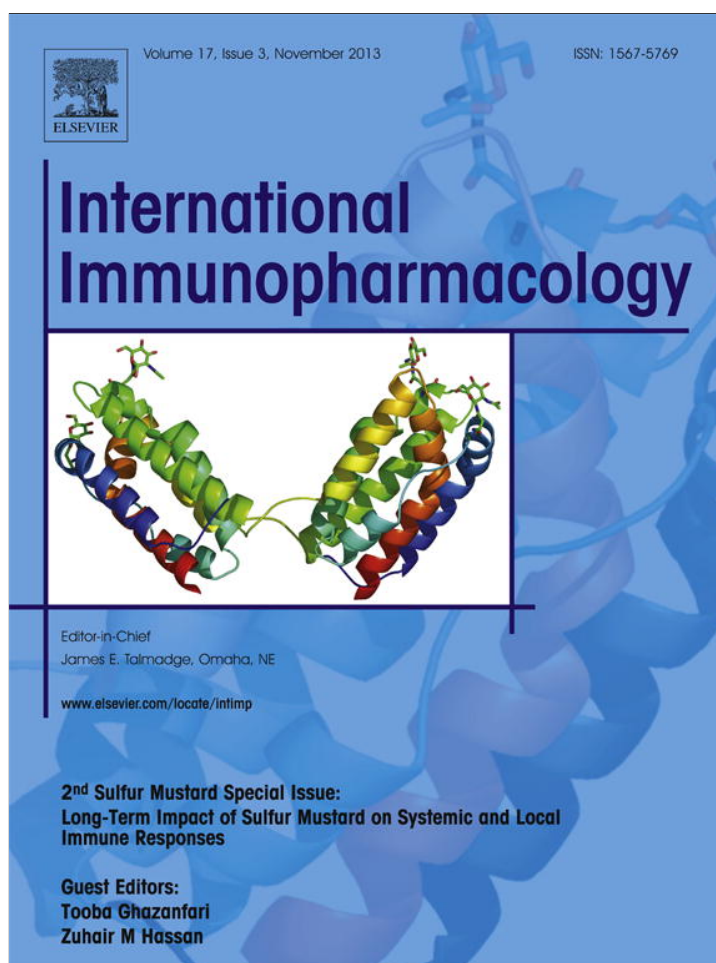


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Association of serum immunoglobulins levels and eye injuries in sulfur mustard exposed: Sardasht-Iran Cohort Study

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ABSTRACT

In this study the associations between ocular problems and serum levels of immunoglobulins in sulfur mustard (SM) exposed population 20 years after exposure in context of Sardasht-Iran Cohort Study was explored. Serum immunoglobulins (Ig) levels including IgM, IgA, IgE, IgG, and subclasses of IgG (IgG1, IgG2, IgG3 and IgG4) in 372 SM-exposed patients were titrated and compared with 128 unexposed controls considering their ocular problems. In exposed patients with tearing and blurring of vision, serum IgM levels were significantly lower than matched controls ($P=0.026$ and 0.027 , respectively). Serum IgM levels in exposed patients with normal ocular conditions were significantly lower ($P<0.050$) than that of matched controls. Serum levels of IgA, IgE and IgG and IgG3 levels were not significantly different between the two groups with abnormal and normal ocular conditions. Mean serum IgG1 levels in exposed patients with normal ocular conditions were significantly higher than the matched controls ($P<0.05$) except for tearing and photophobia. Mean serum IgG2 levels in exposed with blurring of vision and without tearing, ocular pain, photophobia, lids and bulbar conjunctival abnormalities were significantly higher than that of matched controls ($P<0.050$). Mean serum levels of IgG4 in exposed patients with normal ocular conditions and most of the abnormal ocular conditions were significantly lower than the matched controls ($P<0.05$). The results of the current study showed that even 20 years after SM exposure serum immunoglobulins are different from matched normal controls and the levels of IgM and IgG4 are associated with some aspects of ocular surface problems.

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1. Introduction

SM with DNA alkylating activities is the most dangerous vesicant agent. Different organs such as eyes, skin, and lungs, are the main target of SM in liquid or gas forms [1]. Eyes are very sensitive to sulfur mustard (SM) gas because of the wet surfaces. In a large sample of SM casualties,

in the Sardasht-Iran Cohort Study (SICS) photophobia and ocular surface discomfort (burning, itching, and redness) were the most significant symptoms and bulbar conjunctiva and limbal tissue abnormalities were the most significant signs [2,3].

The molecular mechanism(s) involved in sulfur mustard-induced ocular problems is not well known yet. Local and systemic evaluations are necessary to attain a more clear image of the mechanism(s), therefore antibody levels were checked to explore a possible relation of antibody response with ocular problems induced by sulfur mustard [4–11].

Hassan et al. in a short-time follow up on Iranian SM exposed patients showed that in short term follow up, IgM levels initially increased but

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decreased after 6 months. Serum level of IgG was initially higher than normal, but gradually returned to normal within 6 months and IgA levels were high only in less than 10% of the patients. In long term, IgM levels were elevated only in severely affected patients, IgG and IgA levels were normal but mucosal IgA was decreased significantly. IgE levels were high only in about one fifth of the patients [12]. Another study done in chronic phase of severe SM toxicity showed a significantly higher serum IgM level in study group 16 to 20 years after SM exposure [13]. These studies were not focused on the relation between immune responses and ocular complications in SM intoxicated patients.

Like SM, injuries, some ocular surface disorders such as Sjogren syndrome (SS), allergic or vernal keratoconjunctivitis and mucous membrane pemphigoid are examples of simultaneous ocular and immune system involvement. Presence of specific types of circulating immunoglobulin and ocular surface involvement are the characteristic feature of such disorders [14–18].

In previous study we have shown an association between the serum levels of inflammatory mediators and ocular injuries induced by SM [19]. The aim of the current study is to evaluate the possible association between serum immunoglobulin levels and ocular surface disorders in SM intoxicated patients.

2. Materials and methods

2.1. Study design and participants

The details of the design and methodology, including inclusion/exclusion criteria of the study have been previously described in Sardasht Iran Cohort Study (SICS, 2009). No significant difference was seen between the baseline information such as age, body mass index, marital status, and smoking habits of the two groups [2,20]. The age range of the participants was 20–60 years. A total of SM 372 individual after 20 years of exposure were compared with 128 unexposed age/gender matched controls. Special ophthalmic variables related to ocular problems including the patients' complaints were checked using slit-lamp biomicroscopy, and direct and indirect ophthalmoscopy; and then the report was completed by an ophthalmologist. The clinical evaluations were performed during 2007. Based on the previously defined classification for severity of ocular involvement [21], most of the patients were in the category of mild ocular involvement. By this definition, conjunctival vascular dilation, telangiectasia, tortuosity, segmentation, and subconjunctival hemorrhage, were considered as characteristics of the mild group.

2.2. Ethical considerations

The study was approved by the Ethical Committees of Iranian Ministry of Health and Medical Education, Ethics Boards of the Janbazan Medical and Engineering Research Center and Shahed University. The study protocol was performed according to Helsinki Declaration. Potential candidates who were willing to donate samples and sign an informed consent were recruited.

2.3. Clinical evaluation

An ocular examination chart and comprehensive questionnaire were used for every participant. An interviewer recorded the systemic and ocular history in addition to symptoms such as photophobia, ocular surface discomfort (burning, itching, and redness), foreign body sensation, tearing, pain, blurred vision, and dry eye sensation according to the volunteer's complain. A slit lamp biomicroscope (Nidek model, Gamagori, Japan) was used to evaluate the lids, tear meniscus layer, bulbar conjunctiva, limbal tissue, cornea, and anterior segment. Ocular Posterior segments were assessed using direct and indirect ophthalmoscopes (Heine K 180 Ophthalmoscope, Germany

and Heine Omega 100 EN20-1 Binocular Indirect Ophthalmoscope, Germany).

2.4. Serum collection

At the time of clinical evaluations (2007) peripheral blood samples were drawn into Vacutainer tubes (BD Biosciences). Sera were separated via 20 minute centrifugation at 2000 ×g (4 °C), aliquoted, and stored –80 °C and laboratory measurements were performed for 6 months.

2.5. Immunoglobulin measurement

A capture ELISA method was used to titrate immunoglobulin classes and IgG subclasses. Anti-IgG, -IgA, -IgM and -IgE (Bethyl, 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 washing two times using 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 the wells in duplicates 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, 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 incubation in the dark, the reaction was stopped using 100 µl of 5% (v/v) solution of sulfuric acid and read using an ELIZA reader (Awareness, USA) at 450 nm.

2.6. Statistical analysis

Data is presented as mean ± SD. Data analysis was performed using SPSS software, version 16 (SPSS Inc, Chicago, USA). Since the sample size was small in some subgroups, a non-parametric Mann–Whitney test was used to compare the data in the study groups. $P \leq 0.05$ was considered statistically significant. Ocular findings which were positive only in 10 cases or less, were ignored due to low statistical reliability

3. Results

Mean serum IgM level in SM exposed participants with abnormal ocular conditions of tearing and blurring of vision, was significantly lower than that of the matched controls ($P = 0.026$ and 0.027 respectively). Overall, mean serum IgM level in SM exposed participants with normal ocular conditions was significantly lower than the matched controls ($P < 0.05$) (Table 1). No statistical difference was seen in IgM level between SM-exposed participants with normal and abnormal ocular findings except in individuals with tear meniscus abnormality.

Mean serum level of IgA in SM exposed participants with ocular surface discomfort was significantly lower ($P = 0.020$) than the SM-exposed participants with no ocular surface discomfort symptom. Mean serum IgA level in controls and SM-exposed participants with photophobia was significantly higher than in participants with no photophobia in each group ($P = 0.018$ and 0.037 respectively). Overall, in normal and abnormal ocular conditions, mean serum IgA level was not significantly different between the two groups (Table 2).

Mean serum IgE level was not significantly different between the two groups with normal and abnormal ocular conditions, an exception being in SM exposed participants without tearing in which a significantly lower IgE level was seen when compared to that of the matched controls ($P = 0.026$). Mean serum IgE level in controls and those exposed with ocular surface discomfort was significantly higher than in those without this symptom ($P = 0.022$ and 0.035 respectively). Serum IgE

Table 1
Association of the serum levels of IgM with ocular problems in SM induced mild eye injured patients.

Ocular sign and symptoms		IgM (mg/ml)								P-value ²
		Control				Exposed				
		N	Mean	SD	P-value ¹	N	Mean	SD	P-value ¹	
Ocular surface discomfort	No	96	1.512	0.774	0.985	250	1.279	0.733	0.768	0.004
	Yes	25	1.551	0.784		95	1.286	0.631		0.113
Tearing	No	89	1.465	0.705	0.384	230	1.253	0.648	0.517	0.010
	Yes	32	1.672	0.932		115	1.338	0.807		0.026
Dry eye sensation	No	113	1.497	0.752	0.312	328	1.289	0.711	0.570	0.003
	Yes	8	1.840	1.035		17	1.124	0.584		0.062
Pain	No	107	1.470	0.727	0.093	316	1.272	0.707	0.296	0.004
	Yes	14	1.900	1.015		29	1.379	0.690		0.120
Blurred vision	No	76	1.517	0.791	0.725	195	1.278	0.720	0.933	0.013
	Yes	45	1.525	0.750		150	1.285	0.689		0.027
Foreign body sensation	No	121	1.520	0.773		338	1.286	0.710	0.473	0.001
	Yes	0				7	1.021	0.383		
Photophobia	No	96	1.587	0.798	0.053	218	1.302	0.648	0.191	0.001
	Yes	25	1.262	0.617		127	1.244	0.796		0.631
Lids	Normal	118	1.517	0.780	0.424	336	1.277	0.711	0.281	0.001
	Abnormal	3	1.651	0.428		9	1.411	0.480		0.405
Bulbar conjunctive	Normal	119	1.518	0.779	0.502	309	1.270	0.708	0.311	0.001
	Abnormal	2	1.627	0.241		36	1.372	0.685		0.327
Cornea	Normal	120	1.490	0.704	0.086	337	1.276	0.701	0.431	0.001
	Abnormal	1	5.069			8	1.511	0.896		0.121
Tear meniscus abnormality	Normal	111	1.525	0.791	0.970	301	1.251	0.702	0.017	0.000
	Abnormal	10	1.461	0.564		44	1.486	0.700		0.841
Limbus	Normal	121	1.520	0.773		335	1.274	0.701	0.287	0.001
	Abnormal	0				10	1.529	0.836		
Have finding in slit lamp	Normal	107	1.489	0.725	0.364	272	1.252	0.709	0.071	0.001
	Abnormal	14	1.759	1.078		73	1.390	0.686		0.149
Ophthalmic assessment	Normal	113	1.524	0.785	0.851	275	1.248	0.698	0.090	0.000
	Abnormal	8	1.458	0.622		70	1.410	0.724		0.542

P-value¹: Comparison of participants who had ophthalmic finding with those that did not have ophthalmic finding within each study group (Mann–Whitney).

P-value²: Comparison between parallel (marched) exposed and control group (Mann–Whitney).

IgM levels are lower in all exposed group who have normal ocular criteria in compared to the normal corresponding control group. However serum IgM levels in exposed with tearing and blurring of visions were significantly lower than those in the matched controls. Serum IgM levels in exposed with normal ocular conditions were significantly lower than those of the matched controls. No statistical difference was found in IgM levels between exposed with normal and abnormal ocular findings except Tear meniscus abnormality.

level in those exposed with tearing and without corneal abnormality was significantly higher than that of exposed subjects ($P = 0.009$ and 0.039 respectively) (Table 3).

Mean serum IgG level in those exposed with any problem in the final ophthalmic assessment (low material) was significantly lower than that of SM exposed patients without ophthalmic problem (0.048) (Table 4). Overall, in normal and abnormal ocular conditions, mean serum IgG, and IgG3 levels were not significantly different between the two groups (Tables 4–7).

Mean serum IgG1 level in exposed with normal ocular condition in all items was significantly higher than that of the matched control groups ($P < 0.050$) except for those participants with tearing and photophobia (Table 5).

Mean serum IgG2 level in SM exposed participants without tearing, ocular pain, photophobia, lids and bulbar conjunctival abnormalities was significantly higher than that of the matched controls ($P < 0.050$). Mean serum IgG2 level in SM exposed participants with blurring of vision was significantly higher than that of the matched controls ($P = 0.021$). Serum IgG2 level in SM exposed participants with ocular surface discomfort, and foreign body sensation (low material) was significantly lower than that of the SM exposed participants without this symptoms ($P = 0.002$ and 0.021 respectively). Mean serum IgG2 level in control groups with bulbar conjunctival abnormality (low material) was significantly higher than the controls without this finding ($P = 0.022$) (Table 6).

Mean serum IgG4 level in SM exposed participants with normal ocular condition in all items was significantly lower than that of the matched control groups ($P < 0.05$). Mean serum IgG4 level in the exposed participants with ocular problems in all items was significantly lower than that of the matched controls ($P < 0.05$) except for those with foreign body sensation (low material), photophobia, lids (low material),

bulbar conjunctival and corneal (low material), abnormalities. Mean serum IgG4 level in the control groups with photophobia and lids problems (low material) was significantly lower than that of those without these problems ($P = 0.026$ and 0.043 respectively) (Table 8).

4. Discussion

In addition to the local toxic effects of SM on organs such as eyes, lungs, and skin, SM induces serious effects on immune responses. Briefly, the most significant findings of the current study in patients with mild ocular injuries induced by SM toxicity compared to the controls, is serum IgM level which was significantly lower in all normal and in two abnormal ocular conditions including tearing and blurring of vision. The authors proposed that the observed decreased level of IgM in SM-exposed patients is mainly associated to SM toxicity on immune system instead of a direct effect on the ocular condition. Nearly in all normal and abnormal ocular conditions, serum IgA, IgE, IgG and IgG3 levels were not significantly different between the two groups. Mean serum IgG1 level in SM exposed with normal ocular condition in almost all subjects was significantly higher than that of the matched controls. Mean serum IgG2 level in the exposed group without tearing, ocular pain, photophobia, lids and bulbar conjunctival abnormalities was significantly higher than that of the matched controls. Serum IgG4 level in exposed was significantly decreased in all individuals with normal and the most participants with abnormal ocular conditions.

Mahmoudi et al. and Balali-Mood et al. showed that in chronic phase of SM exposure, serum IgM level was significantly higher in severely SM intoxicated patients than that of control groups [13,22]. Hassan et al. also reported that in long term intoxication, IgM level was elevated only in severely affected patients. IgG and IgA levels remained normal, and IgE level was high only in about one fifth of

Table 2
Association of the serum levels of IgA with ocular problems in SM induced mild eye injured patients.

Ocular sign and symptoms		IgA (mg/ml)								P-value ²
		Control				Exposed				
		N	Mean	SD	P-value ¹	N	Mean	SD	P-value ¹	
Ocular surface discomfort	No	96	3.768	1.050	0.458	250	3.877	1.262	0.020	0.831
	Yes	25	3.727	0.775		95	3.507	1.119		0.403
Tearing	No	89	3.806	1.045	0.242	230	3.821	1.260	0.369	0.741
	Yes	32	3.630	0.852		115	3.685	1.181		0.925
Dry eye sensation	No	113	3.776	0.995	0.754	328	3.771	1.231	0.792	0.655
	Yes	8	3.525	1.067		17	3.855	1.328		0.954
Pain	No	107	3.756	1.004	0.549	316	3.796	1.238	0.276	0.953
	Yes	14	3.786	0.973		29	3.553	1.182		0.288
Blurred vision	No	76	3.614	0.963	0.079	195	3.659	1.251	0.056	0.846
	Yes	45	4.004	1.015		150	3.927	1.198		0.527
Foreign body sensation	No	121	3.759	0.997		338	3.769	1.226	0.452	0.641
	Yes	0				7	4.095	1.659		
Photophobia	No	96	3.671	0.966	0.018	218	3.670	1.151	0.037	0.695
	Yes	25	4.097	1.060		127	3.957	1.351		0.387
Lids	Normal	118	3.755	0.996	0.790	336	3.766	1.227	0.424	0.663
	Abnormal	3	3.940	1.227		9	4.138	1.524		0.782
Bulbar conjunctive	Normal	119	3.761	1.003	0.823	309	3.769	1.238	0.584	0.621
	Abnormal	2	3.641	0.667		36	3.827	1.219		0.744
Cornea	Normal	120	3.769	0.995	0.152	337	3.780	1.225	0.880	0.620
	Abnormal	1	2.568			8	3.578	1.666		0.245
Tear meniscus abnormality	Normal	111	3.778	1.023	0.247	301	3.746	1.224	0.128	0.375
	Abnormal	10	3.554	0.633		44	3.976	1.295		0.119
Limbus	Normal	121	3.759	0.997		335	3.768	1.243	0.341	0.636
	Abnormal	0				10	4.011	0.896		
Have finding in slit lamp	Normal	107	3.784	1.021	0.203	272	3.755	1.240	0.435	0.417
	Abnormal	14	3.567	0.786		73	3.851	1.216		0.239
Ophthalmic assessment	Normal	113	3.761	1.008	0.778	275	3.761	1.251	0.464	0.538
	Abnormal	8	3.729	0.886		70	3.832	1.174		0.633

P-value¹: Comparison of participants with Ophthalmic findings with those with no Ophthalmic finding within each study group (Mann–Whitney).

P-value²: Comparison between parallel (marched) exposed and control group (Mann–Whitney).

Table 3
Association of the serum levels of IgE with ocular problems in SM induced mild eye injured patients.

Ocular sign and symptoms		IgE (ng/ml)								P-value ²
		Control				Exposed				
		N	Mean	SD	P-value ¹	N	Mean	SD	P-value ¹	
Ocular surface discomfort	No	95	184.088	256.361	0.022	250	191.523	274.359	0.035	0.237
	Yes	25	337.103	437.381		95	225.996	295.196		0.087
Tearing	No	88	205.159	271.830	0.771	230	176.826	250.522	0.009	0.026
	Yes	32	245.687	392.071		115	249.395	327.670		0.778
Dry eye sensation	No	112	209.901	310.338	0.101	328	200.475	283.425	0.268	0.192
	Yes	8	300.881	261.924		17	211.454	215.951		0.522
Pain	No	106	201.168	261.237	0.987	316	205.616	282.658	0.409	0.179
	Yes	14	328.010	545.454		29	150.887	251.380		0.422
Blurred vision	No	75	230.705	358.882	0.994	195	177.485	241.949	0.825	0.195
	Yes	45	191.402	194.122		150	231.606	321.605		0.408
Foreign body sensation	No	120	215.966	307.230		338	197.950	273.723	0.940	0.119
	Yes	0				7	349.070	517.978		
Photophobia	No	95	231.783	336.995	0.809	218	208.732	289.053	0.556	0.255
	Yes	25	155.861	135.884		127	187.770	265.024		0.415
Lids	Normal	117	219.105	310.229	0.257	336	200.821	280.919	0.608	0.089
	Abnormal	3	93.551	105.359		9	208.285	268.997		0.309
Bulbar conjunctive	Normal	118	214.696	309.152	0.251	309	189.760	263.665	0.073	0.083
	Abnormal	2	290.906	196.709		36	297.628	386.521		0.395
Cornea	Normal	119	217.493	308.071	0.242	337	204.434	282.508	0.039	0.145
	Abnormal	1	34.300			8	57.009	65.076		0.699
Tear meniscus abnormality	Normal	110	213.833	317.961	0.129	301	192.673	264.986	0.321	0.186
	Abnormal	10	239.431	151.505		44	258.085	366.835		0.204
Limbus	Normal	120	215.966	307.230		335	202.627	282.735	0.526	0.135
	Abnormal	0				10	147.043	178.386		
Have finding in slit lamp	Normal	106	218.931	322.479	0.671	272	190.561	262.628	0.262	0.112
	Abnormal	14	193.518	153.221		73	239.969	337.139		0.579
Ophthalmic assessment	Normal	112	217.892	314.441	0.825	275	195.767	267.147	0.791	0.108
	Abnormal	8	189.000	189.458		70	221.638	328.015		0.908

P-value¹: Comparison of participants with Ophthalmic findings with those with no ophthalmic finding within each study group (Mann–Whitney).

P-value²: Comparison between parallel (marched) exposed and control group (Mann–Whitney).

Table 4
Association of the serum levels of IgG with ocular problems in SM induced mild eye injured patients.

Ocular sign and symptoms		IgG (mg/ml)								P-value ²
		Control				Exposed				
		N	Mean	SD	P-value ¹	N	Mean	SD	P-value ¹	
Ocular surface discomfort	No	96	17.403	4.575	0.393	250	17.467	5.012	0.155	0.840
	Yes	25	17.352	2.626		95	16.820	4.550		0.120
Tearing	No	89	17.626	4.382	0.277	230	17.409	4.707	0.775	0.368
	Yes	32	16.742	3.792		115	17.048	5.254		0.639
Dry eye sensation	No	113	17.334	4.348	0.211	328	17.317	4.885	0.401	0.849
	Yes	8	18.217	2.114		17	16.758	5.134		0.200
Pain	No	107	17.329	4.361	0.360	316	17.413	4.962	0.085	0.999
	Yes	14	17.875	3.220		29	15.938	3.862		0.055
Blurred vision	No	76	17.463	4.868	0.780	195	17.138	4.940	0.440	0.535
	Yes	45	17.274	2.923		150	17.485	4.837		0.876
Foreign body sensation	No	121	17.393	4.237		338	17.228	4.864	0.155	0.512
	Yes	0				7	20.211	5.743		
Photophobia	No	96	17.303	4.032	0.946	218	17.431	4.934	0.597	0.772
	Yes	25	17.737	5.024		127	17.046	4.826		0.735
Lids	Normal	118	17.394	4.246	0.920	336	17.262	4.903	0.461	0.543
	Abnormal	3	17.342	4.738		9	18.286	4.579		0.644
Bulbar conjunctive	Normal	119	17.432	4.258	0.300	309	17.413	4.865	0.274	0.662
	Abnormal	2	15.044	1.935		36	16.225	5.060		0.794
Cornea	Normal	120	17.433	4.231	0.129	337	17.350	4.829	0.241	0.577
	Abnormal	1	12.509	.		8	14.739	6.984		0.699
Tear meniscus abnormality	Normal	111	17.405	4.319	0.970	301	17.436	4.943	0.316	0.745
	Abnormal	10	17.257	3.364		44	16.286	4.444		0.462
Limbus	Normal	121	17.393	4.237		335	17.319	4.910	0.406	0.641
	Abnormal	0				10	16.274	4.335		
Have finding in slit lamp	Normal	107	17.452	4.325	0.656	272	17.537	4.975	0.125	0.808
	Abnormal	14	16.936	3.593		73	16.365	4.478		0.619
Ophthalmic assessment	Normal	113	17.381	4.288	0.827	275	17.570	4.946	0.048	0.979
	Abnormal	8	17.554	3.676		70	16.184	4.539		0.323

P-value¹: Comparison of participants with Ophthalmic findings with those with no Ophthalmic finding within each study group (Mann–Whitney).
P-value²: Comparison between parallel (marched) exposed and control group (Mann–Whitney).

Table 5
Association of the serum levels of IgG1 with ocular problems in SM induced mild eye injured patients.

Ocular sign and symptoms		IgG1 (mg/ml)								P-value ²
		Control				Exposed				
		N	Mean	SD	P-value ¹	N	Mean	SD	P-value ¹	
Ocular surface discomfort	No	95	6.817	5.273	0.739	251	8.037	6.137	0.106	0.016
	Yes	25	6.623	5.374		95	7.247	5.386		0.392
Tearing	No	88	6.949	5.417	0.489	230	7.908	6.246	0.932	0.074
	Yes	32	6.302	4.901		116	7.647	5.314		0.107
Dry eye sensation	No	112	6.888	5.387	0.542	329	7.811	5.995	0.742	0.035
	Yes	8	5.209	3.049		17	7.990	4.974		0.221
Pain	No	106	6.827	5.282	0.452	317	7.968	6.075	0.120	0.023
	Yes	14	6.388	5.377		29	6.209	3.971		0.641
Blurred vision	No	76	6.635	4.949	0.875	196	7.561	5.117	0.977	0.047
	Yes	44	7.020	5.839		150	8.159	6.879		0.177
Foreign body sensation	No	120	6.776	5.272		339	7.838	5.987	0.912	0.020
	Yes	0				7	6.966	3.342		
Photophobia	No	95	6.736	5.492	0.294	219	7.463	6.071	0.032	0.089
	Yes	25	6.928	4.432		127	8.435	5.687		0.256
Lids	Normal	117	6.842	5.321	0.496	337	7.831	5.964	0.855	0.026
	Abnormal	3	4.213	1.257		9	7.422	5.417		0.309
Bulbar conjunctive	Normal	118	6.826	5.303	0.424	310	7.826	5.984	0.839	0.024
	Abnormal	2	3.863	0.515		36	7.773	5.663		0.239
Cornea	Normal	119	6.812	5.279	0.170	338	7.827	5.947	0.631	0.021
	Abnormal	1	2.476			8	7.522	6.153		0.245
Tear meniscus abnormality	Normal	110	6.753	5.321	0.500	302	7.754	5.916	0.908	0.017
	Abnormal	10	7.026	4.952		44	8.276	6.181		0.789
Limbus	Normal	120	6.776	5.272		336	7.802	5.934	0.936	0.019
	Abnormal	0				10	8.435	6.550		
Have finding in slit lamp	Normal	106	6.866	5.384	0.889	273	7.676	5.918	0.588	0.046
	Abnormal	14	6.098	4.440		73	8.360	6.048		0.212
Ophthalmic assessment	Normal	112	6.638	5.210	0.303	276	7.740	5.892	0.857	0.010
	Abnormal	8	8.708	6.119		70	8.135	6.175		0.754

P-value¹: Comparison of participants with Ophthalmic findings with those with no Ophthalmic finding within each study group (Mann–Whitney).
P-value²: Comparison between parallel (marched) exposed and control group (Mann–Whitney).

Table 6
Association of the serum levels of IgG2 with ocular problems in SM induced mild eye injured patients.

Ocular sign and symptoms		IgG2 (mg/ml)								P-value ²
		Control				Exposed				
		N	Mean	SD	P-value ¹	N	Mean	SD	P-value ¹	
Ocular surface discomfort	No	95	3.764	2.838	0.052	251	3.868	2.233	0.002	0.073
	Yes	25	4.692	3.030		95	4.505	2.169		0.789
Tearing	No	88	3.819	2.929	0.128	230	4.099	2.299	0.427	0.016
	Yes	32	4.338	2.792		116	3.933	2.096		0.713
Dry eye sensation	No	112	4.022	2.967	0.528	329	4.061	2.240	0.552	0.088
	Yes	8	3.052	1.217		17	3.703	2.090		0.600
Pain	No	106	3.917	2.954	0.364	317	4.049	2.259	0.788	0.039
	Yes	14	4.262	2.436		29	3.974	1.927		0.897
Blurred vision	No	76	3.988	2.539	0.288	196	4.050	2.243	0.834	0.458
	Yes	44	3.904	3.447		150	4.034	2.223		0.021
Foreign body sensation	No	120	3.957	2.891		339	4.009	2.224	0.021	0.080
	Yes	0				7	5.694	2.052		
Photophobia	No	95	3.971	2.955	0.979	219	4.217	2.339	0.083	0.019
	Yes	25	3.905	2.689		127	3.743	2.004		0.870
Lids	Normal	117	3.959	2.919	0.699	337	4.074	2.240	0.094	0.039
	Abnormal	3	3.883	1.716		9	2.899	1.574		0.309
Bulbar conjunctive	Normal	118	3.831	2.742	0.022	310	4.061	2.289	0.855	0.032
	Abnormal	2	11.392	1.567		36	3.889	1.667		0.019
Cornea	Normal	119	3.979	2.894	0.145	338	4.056	2.249	0.562	0.070
	Abnormal	1	1.404			8	3.479	1.151		0.121
Tear meniscus abnormality	Normal	110	3.917	2.791	0.909	302	3.968	2.150	0.117	0.116
	Abnormal	10	4.401	3.990		44	4.555	2.696		0.295
Limbus	Normal	120	3.957	2.891		336	4.038	2.232	0.860	0.060
	Abnormal	0				10	4.205	2.295		
Have finding in slit lamp	Normal	106	3.942	2.823	0.747	273	3.987	2.197	0.314	0.129
	Abnormal	14	4.076	3.480		73	4.251	2.357		0.248
Ophthalmic assessment	Normal	112	3.868	2.790	0.371	276	3.959	2.198	0.093	0.094
	Abnormal	8	5.202	4.091		70	4.376	2.345		0.882

P-value¹: Comparison of participants with ophthalmic findings with those with no Ophthalmic finding within each study group (Mann–Whitney).
P-value²: Comparison between parallel (marched) exposed and control group (Mann–Whitney).

Table 7
Association of the serum levels of IgG3 with ocular problems in SM induced mild eye injured patients.

Ocular sign and symptoms		IgG3 (mg/ml)								P-value ²
		Control				Exposed				
		N	Mean	SD	P-value ¹	N	Mean	SD	P-value ¹	
Ocular surface discomfort	No	79	0.817	0.484	0.246	243	0.877	0.408	0.450	0.149
	Yes	21	0.882	0.340		81	0.856	0.443		0.401
Tearing	No	74	0.819	0.483	0.432	215	0.868	0.419	0.946	0.265
	Yes	26	0.864	0.378		109	0.879	0.414		0.896
Dry eye sensation	No	93	0.831	0.470	0.646	308	0.879	0.423	0.264	0.267
	Yes	7	0.824	0.245		16	0.726	0.213		0.423
Pain	No	88	0.837	0.482	0.722	296	0.885	0.426	0.135	0.248
	Yes	12	0.785	0.193		28	0.737	0.270		0.516
Blurred vision	No	62	0.804	0.339	0.879	181	0.838	0.379	0.203	0.855
	Yes	38	0.874	0.605		143	0.915	0.458		0.259
Foreign body sensation	No	100	0.830	0.457		317	0.870	0.418	0.502	0.386
	Yes	0				7	0.936	0.390		
Photophobia	No	76	0.814	0.483	0.323	203	0.866	0.417	0.664	0.289
	Yes	24	0.883	0.369		121	0.881	0.418		0.754
Lids	Normal	98	0.831	0.462	0.999	317	0.869	0.414	0.431	0.383
	Abnormal	2	0.779	0.127		7	1.002	0.553		0.558
Bulbar conjunctive	Normal	99	0.836	0.455	0.100	289	0.868	0.423	0.237	0.573
	Abnormal	1	0.221			35	0.905	0.364		0.092
Cornea	Normal	99	0.824	0.456	0.141	316	0.871	0.422	0.254	0.345
	Abnormal	1	1.411			8	0.895	0.112		0.121
Tear meniscus abnormality	Normal	92	0.833	0.467	0.770	287	0.864	0.414	0.308	0.436
	Abnormal	8	0.795	0.347		37	0.934	0.435		0.635
Limbus	Normal	100	0.830	0.457		315	0.872	0.414	0.666	0.344
	Abnormal	0				9	0.863	0.543		
Have finding in slit lamp	Normal	89	0.828	0.470	0.471	260	0.861	0.422	0.154	0.454
	Abnormal	11	0.848	0.347		64	0.915	0.396		0.893
Ophthalmic assessment	Normal	93	0.840	0.466	0.539	263	0.859	0.419	0.139	0.695
	Abnormal	7	0.696	0.309		61	0.927	0.407		0.200

P-value¹: Comparison of participants with ophthalmic findings with those with no ophthalmic finding within each study group (Mann–Whitney).
P-value²: Comparison between parallel (marched) exposed and control group (Mann–Whitney).

Table 8
Association of the serum levels of IgG4 with ocular problems in SM induced mild eye injured patients.

Ocular sign and symptoms		IgG4 (mg/ml)								P-value ²
		Control				Exposed				
		N	Mean	SD	P-value ¹	N	Mean	SD	P-value ¹	
Ocular surface discomfort	No	94	0.695	0.152	0.223	250	0.668	0.303	0.154	0.027
	Yes	25	0.781	0.250		95	0.623	0.196		0.003
Tearing	No	87	0.692	0.156	0.135	229	0.665	0.315	0.715	0.030
	Yes	32	0.769	0.224		116	0.637	0.185		0.003
Dry eye sensation	No	111	0.712	0.185	0.644	328	0.658	0.282	0.346	0.002
	Yes	8	0.724	0.045		17	0.611	0.189		0.031
Pain	No	105	0.705	0.187	0.061	316	0.657	0.285	0.910	0.006
	Yes	14	0.772	0.088		29	0.643	0.191		0.016
Blurred vision	No	75	0.700	0.199	0.134	195	0.635	0.198	0.267	0.017
	Yes	44	0.735	0.138		150	0.682	0.356		0.011
Foreign body sensation	No	119	0.713	0.179		338	0.656	0.280	0.860	0.001
	Yes	0				7	0.613	0.210		
Photophobia	No	94	0.732	0.173	0.026	219	0.668	0.304	0.170	0.001
	Yes	25	0.642	0.186		126	0.634	0.228		0.674
Lids	Normal	116	0.718	0.179	0.043	336	0.658	0.281	0.180	0.000
	Abnormal	3	0.536	0.042		9	0.558	0.159		0.926
Bulbar conjunctive	Normal	117	0.713	0.178	0.967	309	0.655	0.289	0.544	0.001
	Abnormal	2	0.718	0.331		36	0.658	0.168		0.744
Cornea	Normal	118	0.711	0.179	0.211	337	0.654	0.279	0.287	0.001
	Abnormal	1	0.888			8	0.733	0.227		0.437
Tear meniscus abnormality	Normal	109	0.705	0.176	0.108	301	0.659	0.292	0.624	0.005
	Abnormal	10	0.803	0.200		44	0.632	0.157		0.009
Limbus	Normal	119	0.713	0.179		335	0.656	0.281	0.910	0.001
	Abnormal	0				10	0.648	0.201		
Have finding in slit lamp	Normal	105	0.708	0.176	0.450	272	0.661	0.302	0.679	0.005
	Abnormal	14	0.752	0.205		73	0.635	0.166		0.047
Ophthalmic assessment	Normal	112	0.705	0.174	0.058	275	0.661	0.302	0.786	0.005
	Abnormal	7	0.843	0.228		70	0.635	0.152		0.012

P-value¹: Comparison of participants with Ophthalmic findings with those with no Ophthalmic finding within each study group (Mann–Whitney).
P-value²: Comparison between parallel (marched) exposed and control group (Mann–Whitney).

the patients [12]. In the present study, serum level of IgM in all abnormal ocular conditions, except for the symptoms (and not for the signs) of tearing and blurring of vision, was not significantly different between the two groups. The milder severity and more chronic toxicity in the subjects might be the reason for this discrepancy with the other studies. Alteration in serum IgG, IgA and IgM levels in the first month of exposure to SM has previously been reported by Keyhani et al. in which the IgG level was significantly lower on day 3 post exposure but increased from day 4 and became highly significant during days 19–31. Alterations in serum IgA and IgG levels were similar; however these alterations were not significant. Alterations in serum IgM level were not significantly different from the normal controls. The authors concluded that the initial lower serum IgG level might be due to possible leakage of IgG into the skin blisters or other parts of the body such as the respiratory system, whereas its subsequent increase might be due to later immune systems response [23]. In contrast to IgM and IgG4, serum IgG1 and IgG2 levels in most of the exposed individuals with normal ocular conditions were significantly higher. The differences might be due to the fact that the study was performed a long time after the exposure.

In dry eye induced by SS, circulating monoclonal IgG (primary SS), or IgM (secondary SS) levels are increased [24,25]. Mikulicz's disease, a subtype SS, involving lacrimal and salivary glands is associated with elevated serum immunoglobulin IgG4 [26]. Unlike SS and Mikulicz's disease the findings of this study showed that in SM exposed, serum IgM level in all normal and two abnormal ocular conditions of tearing and blurring of vision was significantly lower than that of the controls. Our findings suggest that the immune responses in SM exposed patients may be somehow different from that of those in primary SS. In vernal keratoconjunctivitis, a Th2 allergic response, total serum IgE level is significantly higher [27,28]. In the present study ocular surface discomfort caused a significant elevation in serum IgE level in the

control group and in SM exposed, but the differences between the two groups were not significant. Overall, in normal and abnormal ocular conditions, serum IgE level was not different between the two groups. These findings may exclude the allergic mechanisms in SM induced pathology. In mucous membrane pemphigoid, circulating IgG autoantibodies play an important role in the pathogenesis of the disease [29]. In contrast, the results of the present study showed that in normal and abnormal ocular conditions, serum IgG level was not significantly different between the exposed and controls groups, and even in most normal and abnormal ocular conditions, serum IgG4 level was significantly lower than that of the controls. This difference again may contribute to the different immune responses in these patients.

5. Conclusion

The results of this study showed that in patients with chronic SM toxicity, alterations in serum immunoglobulins especially IgM and IgG4 levels are at least associated with some aspects of ocular surface problems. However, importantly these findings showed that a single episode of exposure could affect immune parameters in SM-intoxicated people long term after the exposure, even with no clinical complication or problem.

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