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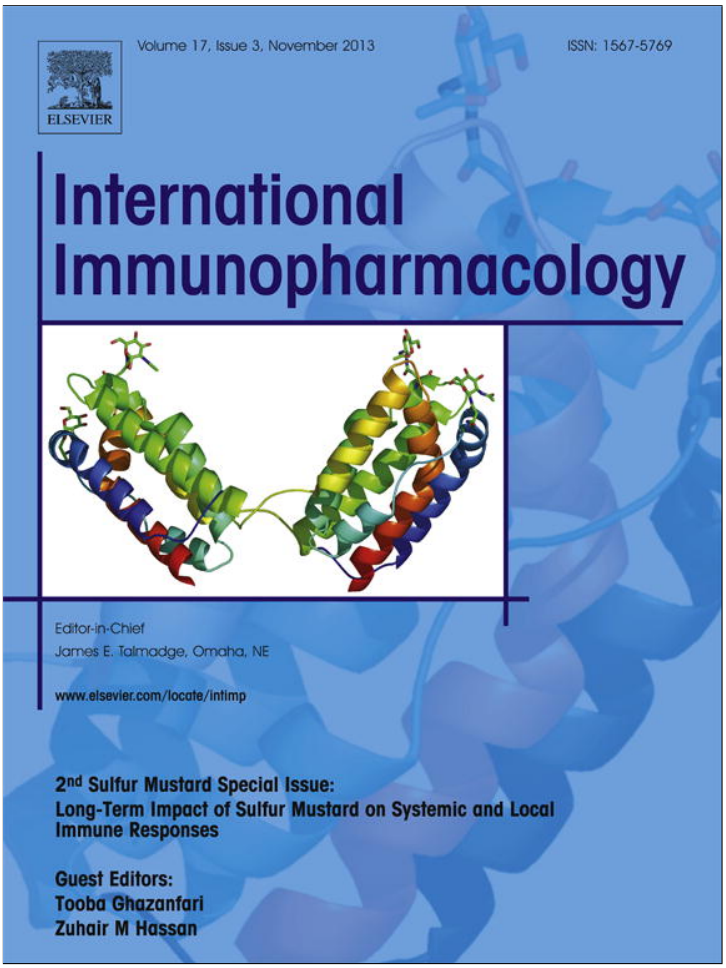

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Association of ophthalmic complications in patients with sulfur mustard induced mild ocular complications and serum soluble adhesion molecules: Sardasht–Iran Cohort Study

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ABSTRACT

The aim of this study was to evaluate possible association between ophthalmic complications in sulfur mustard (SM) exposed patients with mild ocular injuries and serum soluble adhesion molecules.
Serum levels of sICAM-1, sL-selectin, sP-selectin and sE-selectin in 367 SM-exposed individuals with or without eye injuries were checked and compared with 128 unexposed controls. All participants underwent ocular examinations.
Serum sICAM-1 level in SM exposed with blurred vision, was significantly ($p = 0.021$) higher than in SM exposed with no blurred vision. Serum sL-selectin level was significantly ($p = 0.024$) higher in SM exposed with photophobia than SM exposed with no photophobia.
Serum P-selectin level in exposed without any slit lamp findings was significantly ($p = 0.003$) lower than the matched control groups. Similar finding was seen in exposed group without ocular problem compared with the control groups. Serum sE-selectin level in exposed with normal ocular condition except for photophobia and blurred vision was significantly ($p < 0.05$) higher than the matched controls. Serum E-selectin level in exposed with photophobia condition was significantly ($p = 0.047$) higher than the control group with photophobia.
In conclusion it seems that the changes in the E- and P-selectins is a regulatory mechanism for inhibition of SM induced ocular problems, although the local levels are more important and further investigations required in more severe ocular problems in SM exposed patients.

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1. Introduction

Adhesion molecules are glycoproteins which allow cell-to-cell contact, control of immune surveillance and the function of effector cells. Selectins are a family of carbohydrate-binding proteins involved in a wide variety of interactions between leukocytes and endothelial cells [1]. Plasma soluble selectins include platelet activation-dependent granule-external membrane protein (P-Selectin), endothelial leukocyte adhesion molecule (E-Selectin), and leukocyte endothelial cell adhesion molecule-1 (L-Selectin) [2]. Apart from selectins, two other members of adhesion molecules are immunoglobulin super family (intercellular adhesion molecule 1 or ICAM-1, ICAM-2 and vascular cell adhesion molecule 1 or VCAM-1) and the integrins [3]. The selectin family as a group of early-reactive adhesion molecules plays a role in the rolling phase of leukocytes in cellular infiltration. P-selectin contributes to the initial phase of ocular inflammation while E-selectin contributes to continue cellular infiltration at the site of inflammation [4].

Selectins play a different role in the ocular surface disorders. In ocular allergic disease [5] and Mooren ulcer [6], E-selectin and ICAM-1 up-regulate in conjunctiva of patients. ICAM-1 expressed in graft rejection,

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Table 1
Associations between the serum sICAM-1 level and ocular problems in studied groups.

Ocular problems		sICAM-1 (pg/ml)								
		Control				Exposed				p-value[2]
		N	Mean	SD	p-value[1]	N	Mean	SD	p-value[1]	
Ocular surface discomfort	No	98	373.41	147.59	0.424	253	394.90	147.87	0.114	0.565
	Yes	25	420.95	159.87		98	357.77	94.78		0.180
Tearing	No	91	377.00	156.83	0.848	234	373.48	120.66	0.155	0.997
	Yes	32	400.34	132.59		117	406.64	160.83		0.996
Dry eye sensation	No	115	385.62	152.97	0.871	334	385.39	135.25	0.958	0.999
	Yes	8	346.50	114.90		17	367.78	154.55		0.985
Pain	No	109	387.56	153.76	0.755	322	385.50	137.34	0.973	0.999
	Yes	14	348.16	123.65		29	373.76	122.50		0.943
Blurred vision	No	77	367.27	125.47	0.360	201	366.02	119.67	**0.021**	0.999
	Yes	46	409.52	184.00		150	409.34	152.24		0.999
Foreign body sensation	No	123	383.07	150.72		344	384.99	136.41	0.904	0.991
	Yes					7	362.09	124.44		
Photophobia	No	97	397.95	160.77	0.103	223	386.21	136.63	0.991	0.900
	Yes	26	327.58	86.82		128	381.61	135.53		0.274
Palperbrea (lids)	No	120	382.33	151.15	0.982	342	386.36	136.87	0.433	0.993
	Yes	3	412.93	157.75		9	315.04	75.62		0.720
Bulbar conjunctiva	No	121	383.50	151.86	0.994	315	381.44	132.65	0.612	0.999
	Yes	2	357.10	50.20		36	411.61	162.53		0.950
Cornea	No	122	382.09	150.94		343	382.28	132.88	0.117	0.999
	Yes	1	502.80			8	481.38	227.93		
Tear meniscus	No	113	385.36	153.87	0.929	306	384.05	139.43	0.998	0.999
	Yes	10	357.22	111.76		45	387.80	111.65		0.924
Limbus tissue	No	123	383.07	150.72		341	384.39	137.73	0.993	0.996
	Yes					10	389.52	56.47		
Any slit lamp findings	No	109	383.52	154.72	0.999	277	381.55	133.72	0.868	0.999
	Yes	14	379.55	119.52		74	395.69	144.87		0.979
Any ocular problems	No	115	382.94	153.76	0.999	280	381.83	138.69	0.890	0.999
	Yes	8	384.98	104.22		71	395.21	125.45		0.997

The serum level of sICAM-1 was assessed using ELISA method in the SM exposed and control groups and compared with the volunteers with and without ocular problems within the group and between the SM exposed and the control groups.
p-value[1]: Comparison of the serum level of sICAM-1 between the participants with and without ocular problems within each group (ANOVA, Tukey post hoc).
p-value[2]: Comparison of the serum level of sICAM-1 between the SM exposed and the control groups (ANOVA, Tukey post hoc).

chemical burns and keratitis while E-selectin expressed in limbal vessels in corneas with bacterial keratitis [7]. E-selectin induces corneal endothelial cells chemotaxis and stimulates angiogenesis *in vitro* [8]. P-selectin is an important mediator in corneal inflammation and therefore might be used as a potential target for immunotherapy [9,10]. Platelet and P-selectin localization in the limbus of abraded corneas provides a necessary step in corneal healing process [11]. While selectins are expressed in the sclera and conjunctiva in scleritis, normal human sclera does not express ICAM-1 or E-selectin [12].

Some aspects of immune system effects of SM have been described previously [13–15] and alteration of serum soluble selectins has been reported by the same group [14]. To our knowledge, there is no report on the association between serum level of selectins and ocular problems in SM exposed population. The aim of this study was to evaluate the possible association between the serum levels of ICAM-1, sL-selectin, sP-selectin, and sE-selectin with sulfur mustard induced mild ocular injuries in Sardasht–Iran Cohort Study.

2. Materials and methods

2.1. Study design and participants

The details of the design and methodology including inclusion/exclusion criterion of the study population has been previously described in a Sardasht–Iran Cohort Study (SICS) [16]. The study population includes 372 volunteers with history of SM exposure in June 1997 and as control 128 volunteers with no history of SM exposure and no significant difference in age, body mass index, marital status, and smoking habits were recruited [16]. Based on the previously defined classification of the severity of ocular involvement and changes in conjunctival vessels including dilation, telangiectasia, tortuosity, segmentation, and subconjunctival hemorrhage were defined as the characteristics of the mild group [17]. The clinical evaluation and samples collection were done in June 2007. The study was approved by the Iranian Ministry of Health, Shahed University, and the Ethics Board of the Janbazan Medical and Engineering Research Center. The study protocol followed the ethical frame woks of Declaration of Helsinki. Written informed consent was obtained from every included subject.

2.2. Clinical evaluation

A comprehensive questionnaire and examination chart were developed and completed for every participant. The interviewer and physician recorded 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. A slit lamp biomicroscope (Nidek model, Gamagori, Japan) was used for evaluation of the lids, tear meniscus, bulbar conjunctiva, limbal tissue, cornea, and anterior segment. Posterior segment was evaluated using direct and indirect ophthalmoscopes (Heine K 180 ophthalmoscope, Germany and Heine Omega 100 EN20-1 binocular indirect ophthalmoscope, Germany).

2.3. Serum collection

Peripheral blood sample was drawn into Vacutainer tubes (BD Biosciences). Serum was separated via 20 min centrifugation at 2000 $\times g$ (4 °C), aliquoted, and kept frozen at −80 °C until use.

2.4. Adhesion molecules measurement

Human sICAM-1/CD54, sL-selectin/CD62L, sE-selectin/CD62E, and sP-selectin/CD62P Quantikine® ELISA kits (R&D Systems) were used

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to measure the levels of the adhesion molecules in the sera in accordance with the manufacturer's instructions. The detection limit of the kits was between 1 and 2 pg/ml.

2.5. Statistical analysis

The markers between the following 4 groups were compared; 1. SM Exposed with ocular problems, 2. SM Exposed with no ocular problem, 3. Unexposed control with ocular problems, and 4. Unexposed control with no ocular problems. Data was presented by mean ± standard deviation. Data analysis was performed using SPSS, version 16 (SPSS Inc, Chicago, USA), ANOVA test flowed by Tukey–Kramer post hoc test was used to compare the study groups. Differences were considered statistically significant when p was ≤0.05.The data collected from group with less than 10 participants was not included in the analyses due to small sample size and low statistical reliability.

3. Results

A comparison of the serum levels of sICAM-1/CD54, sL-selectin/CD62L, sE-selectin/CD62E, and sP-selectin/CD62P were undertaken between participants with and without ocular problems (symptoms or signs which were indicated in the clinical evaluation in the materials and method section.

3.1. Comparison of serum level of sICAM between the study groups

Mean serum sICAM-1 level in SM exposed group with blurred vision was significantly ($p<0.05$) higher than the exposed without blurred vision ($p=0.021$). This difference was not significant in the control groups; volunteers with blurred vision *vs.* without blurred vision ($p=0.360$). There was no significant difference in the serum level of sICAM-1 between the SM exposed group with and without other ocular problems. The similar results were seen in the control subgroups. In addition, no significant difference was seen between SM exposed subgroups (with or without ocular problems) and the matched control group (Table 1).

3.2. Comparison of serum level of sL-selectin between study groups

Mean serum sL-selectin level in SM exposed group with photophobia was significantly ($p<0.05$) higher than SM exposed with no photophobia ($p=0.024$), but this difference was not significant in the matched controls with photophobia *vs.* with no photophobia ($p=0.893$). There was no significant difference in the serum level of sL-selectin between the SM exposed group with and without other ocular problems. The similar results were seen in the control subgroups. In addition, no significant difference was seen between SM exposed subgroups (with or without ocular problems) and the matched control groups (Table 2).

3.3. Comparison of serum levels of sP-selectin between study groups

Mean serum sP-selectin level in SM exposed group with abnormal limbal tissue was significantly ($p=0.00$) higher than the exposed without abnormal limbal tissue ($p=0.005$). Serum level of sP-selectin in SM exposed group without any ocular problems was significantly $p<0.001$ lower than the control group without any ocular problems (Table 3).

3.4. Comparison of serum levels of sE-selectin between study groups

Serum sE-selectin level in exposed with photophobia was significantly ($p<0.05$) higher than the matched control groups. Serum level

Table 2
Associations between the serum sL-selectin level and the ocular problems in studied groups.

Ocular problems		sL-selectin (pg/ml)								
		Control				Sulfur mustard exposed				p-value[2]
		N	Mean	SD	p-value[1]	N	Mean	SD	p-value[1]	
Ocular surface discomfort	No	98	12.16	2.97	0.962	252	11.52	3.05	0.773	0.301
	Yes	25	11.82	3.31		98	11.17	3.15		0.777
Tearing	No	91	12.19	3.14	0.930	233	11.50	3.11	0.912	0.265
	Yes	32	11.81	2.71		117	11.27	3.01		0.816
Dry eye sensation	No	115	12.01	2.99	0.704	333	11.43	3.10	0.999	0.291
	Yes	8	13.22	3.49		17	11.35	2.69		0.487
Pain	No	109	12.22	2.92	0.573	321	11.41	3.05	0.998	0.085
	Yes	14	11.10	3.71		29	11.52	3.40		0.974
Blurred vision	No	77	12.05	2.93	0.997	200	11.47	3.06	0.986	0.503
	Yes	46	12.16	3.22		150	11.36	3.10		0.403
Foreign body sensation	No	123	12.09	3.03		343	11.45	3.09	0.389	0.118
	Yes	0	.	.		7	9.92	1.72		
Photophobia	No	97	11.99	2.78	0.893	222	11.07	3.13	**0.024**	0.064
	Yes	26	12.46	3.85		128	12.03	2.89		0.911
Palperbrea (lids)	No	120	12.14	3.05	0.674	341	11.42	3.08	0.999	0.126
	Yes	3	10.12	0.69		9	11.33	2.99		0.935
Bulbar conjunctiva	No	121	12.09	3.04	0.999	314	11.49	3.12	0.657	0.255
	Yes	2	12.04	2.37		36	10.86	2.62		0.953
Cornea	No	122	12.11	3.03		342	11.43	3.10	0.947	0.087
	Yes	1	9.01	.		8	11.08	2.02		
Tear meniscus	No	113	12.11	3.05	0.994	305	11.45	3.15	0.954	0.212
	Yes	10	11.85	2.82		45	11.20	2.54		0.930
Limbus tissue	No	123	12.09	3.03		340	11.38	3.09	0.367	0.074
	Yes	0	.	.		10	12.71	2.42		
Any slit lamp findings	No	109	12.19	3.08	0.716	276	11.53	3.20	0.612	0.217
	Yes	14	11.28	2.56		74	11.03	2.54		0.993
Any ocular problems	No	115	12.18	3.06	0.580	279	11.48	3.17	0.909	0.162
	Yes	8	10.75	2.15		71	11.20	2.66		0.979

The serum level of sL-selectin was assessed using ELISA method in the SM exposed and the control groups and compared within the groups and between the SM exposed and the control groups.
p-value[1]: Comparison of the serum level of sL-selectin between the participants with and without ocular problems within each group (ANOVA, Tukey post hoc).
p-value[2]: Comparison of the serum level of sL-selectin between the SM exposed and the control groups (ANOVA, Tukey post hoc).
Bold data shows significant differences with p value <0.05.

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of sE-selectin in SM exposed without any ocular problems was significantly $p<0.009$ higher than the matched control group. (Table 4).

4. Discussion

The aim of this study was to explore possible association between the serum soluble adhesion molecules with mild ocular complications in a SM exposed population.

Findings of this study showed that there is no significant difference in the serum level of sICAM-1 and sL-selectin between SM exposed group with and without ocular problems except serum sICAM-1 level in the SM exposed group with blurred vision which was significantly higher than the level in exposed without blurred vision and serum sL-selectin level was significantly higher in SM exposed with photophobia than the exposed without this ocular problem. In addition, no significant difference was seen between SM exposed subgroups (with or without ocular problems) and the matched control groups.

It has been previously reported by the same group that serum sL-selectin significantly was lower in SM-exposed individuals compared with unexposed controls [14]. Herein, when the SM-exposed and the control groups were divided into two subgroups, no difference was seen, one reason might be a small sample size.

Mean serum sP-selectin level in exposed group with abnormal limbal tissue was significantly higher than the exposed group without abnormal limbal tissue. Serum level of sP-selectin in SM exposed group without ocular problems was significantly lower than the control group without ocular problems. It has been previously reported by the same group that serum sP-selectin was lower in SM-exposed individuals compared with the unexposed control groups [14]. Serum sE-selectin level in exposed with photophobia was significantly higher than that of the matched controls. Serum level of sE-selectin in SM exposed without ocular problems was significantly higher than that of matched control group. It has been previously reported by the same group that serum sE-selectin was higher or increases in SM-exposed individuals compared with unexposed controls [14]. When the SM-exposed and controls were divided into two subgroups based on the presence or absence of ocular problems, the difference in sP-selectin and sE-selectin levels were only seen in SM exposed group who did not show ocular problem compared to the matched control groups. It seems that the decreased level of sP-selectin and an increased sE-selectin are defense mechanisms.

The current results also revealed that some parts of the immune system are suppressed in SM exposed patients which is in accordance with the findings of other studies [18,19]. Hassan et al. [20] described that SM induces hematological complications and severe suppression of the immune system. Gerecke et al. [21] also showed that cytokine–cytokine receptor interactions, cell adhesion molecules (CAMs), and hematopoietic cell lineage are common pathways affected at different times following SM exposure.

As shown in this study, changes in the serum levels of adhesion molecules related to ocular problems may be due to SM exposure. There is a report which showed a special effect on adhesion molecules as a result of SM exposure but only in animal models with a variety of proposed mechanisms. Chang et al. [22] showed that SM exposure induce increase of mRNA levels for laminin-gamma2 which suggested a potential role in wound healing. Laminin functions as a ligand for integrins and regulates cell adhesion, migration, and morphogenesis. Jin et al. [23] showed that SM induced degradation of attachment proteins like laminin-5 and its two subunits beta3 and gamma2. Werrlein and Marden-Whalley et al. [24] suggested that the alkylating function of SM disrupted adhesion complex molecules and associated signaling mechanisms required for the maintenance and repair. Acute exposure to SM caused a significant decrease in expression of beta-4

Table 3
Associations between the serum sP-selectin level and ocular problems in studied groups.

Ocular problems		sP-selectin (pg/ml)								
		Control				Exposed				p-value[2]
		N	Mean	SD	p-value[1]	N	Mean	SD	p-value[1]	
Ocular surface discomfort	No	98	174.00	61.27	0.598	253	146.58	48.50	0.200	<**0.001**
	Yes	25	159.44	44.41		98	158.83	53.31		0.999
Tearing	No	91	171.37	59.26	0.999	234	151.70	49.83	0.828	**0.013**
	Yes	32	170.09	56.68		117	146.61	50.71		0.113
Dry eye sensation	No	115	170.00	58.22	0.839	334	150.58	49.79	0.799	**0.004**
	Yes	8	185.94	62.62		17	138.72	56.61		0.154
Pain	No	109	171.85	58.49	0.964	322	150.89	50.49	0.714	**0.002**
	Yes	14	164.72	59.19		29	140.11	45.38		0.473
Blurred vision	No	77	175.91	62.77	0.542	201	149.16	53.09	0.985	**0.001**
	Yes	46	162.88	49.75		150	151.13	45.97		0.544
Foreign body sensation	No	123	171.04	58.37		344	150.42	50.49	0.541	**0.001**
	Yes					7	129.28	16.09		
Photophobia	No	97	172.18	58.59	0.966	223	152.49	53.58	0.642	**0.011**
	Yes	26	166.75	58.50		128	145.66	43.27		0.242
Palperbrea (lids)	No	120	171.46	58.89	0.943	342	149.49	49.91	0.672	**0.001**
	Yes	3	154.26	32.62		9	169.47	56.96		0.972
Bulbar conjunctiva	No	121	170.08	58.37	0.393	315	150.09	50.19	0.999	**0.002**
	Yes	2	228.90	5.52		36	149.20	50.15		0.156
Cornea	No	122	171.17	58.59		343	149.41	49.97	0.349	<**0.001**
	Yes	1	154.22	.		8	175.39	53.10		
Tear meniscus	No	113	168.47	57.56	0.261	306	148.70	49.42	0.617	**0.004**
	Yes	10	199.98	62.86		45	158.84	54.36		0.111
Limbal tissue	No	123	171.04	58.37		341	148.51	49.08	**0.005**	<**0.001**
	Yes					10	200.87	60.69		
Any slit lamp findings	No	109	169.00	58.38	0.623	277	148.37	49.89	0.672	**0.003**
	Yes	14	186.92	57.96		74	156.10	50.81		0.182
Any ocular problems	No	115	170.32	58.47	0.939	280	148.61	50.15	0.758	**0.001**
	Yes	8	181.36	59.80		71	155.47	49.92		0.548

The serum level of sP-selectin was assessed using ELISA method and compared within the groups and between the SM exposed and the control groups.
p-value[1]: Comparison of the serum level of sP-selectin between participants with and without ocular problems within each group (ANOVA, Tukey post hoc).
p-value[2]: Comparison of the serum level of sP-selectin between the SM exposed and the control groups (ANOVA, Tukey post hoc).
Bold data shows significant differences with p value <0.05.

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Table 4
Associations between the serum sE-selectin level and ocular problems in studied groups.

Ocular problems		sE-selectin (pg/ml)								
		Control				Exposed				p-value[2]
		N	Mean	SD	p-value[1]	N	Mean	SD	p-value[1]	
Ocular surface discomfort	No	98	25.21	14.00	0.904	253	31.14	17.27	0.224	**0.016**
	Yes	25	22.66	15.23		98	27.33	18.08		0.598
Tearing	No	91	24.72	14.62	0.999	234	29.52	18.18	0.816	0.096
	Yes	32	24.64	13.31		117	31.19	16.24		0.206
Dry eye sensation	No	115	24.76	14.32	0.998	334	30.21	17.67	0.910	**0.015**
	Yes	8	23.77	13.75		17	27.43	15.35		0.957
Pain	No	109	25.29	14.40	0.693	322	30.36	17.95	0.717	**0.033**
	Yes	14	20.08	12.38		29	26.93	12.07		0.591
Blurred vision	No	77	25.12	14.92	0.984	201	30.23	18.02	0.997	0.106
	Yes	46	23.99	13.13		150	29.87	16.97		0.162
Foreign body sensation	No	123	24.70	14.23		344	30.11	17.64	0.969	**0.006**
	Yes					7	28.59	13.59		
Photophobia	No	97	25.26	14.81	0.885	223	29.00	16.90	0.389	0.258
	Yes	26	22.57	11.82		128	31.94	18.56		**0.047**
Palperbrea (lids)	No	120	24.60	14.13	0.976	342	30.12	17.64	0.990	**0.011**
	Yes	3	28.67	21.28		9	28.37	14.87		0.999
Bulbar conjunctiva	No	121	24.70	14.23	0.999	315	29.77	17.43	0.742	**0.025**
	Yes	2	24.53	20.07		36	32.76	18.67		0.906
Cornea	No	122	24.69	14.29		343	29.94	17.43	0.602	**0.009**
	Yes	1	25.75			8	35.71	22.85		
Tear meniscus	No	113	24.39	14.16	0.907	306	30.63	17.76	0.375	**0.004**
	Yes	10	28.12	15.44		45	26.33	15.72		0.990
Limbus tissue	No	123	24.70	14.23		341	30.15	17.62	0.879	**0.006**
	Yes					10	27.55	15.83		
Any slit lamp findings	No	109	24.26	14.10	0.855	277	30.50	18.05	0.793	**0.006**
	Yes	14	28.07	15.34		74	28.48	15.59		0.999
Any ocular problems	No	115	24.62	14.11	0.998	280	30.50	18.18	0.788	**0.009**
	Yes	8	25.76	16.87		71	28.42	14.85		0.974

The serum level of sE-selectin was assessed by ELISA method in the SM exposed and the control groups and compared with those with and without ocular problems within the groups and between the SM exposed and control groups.
p-value[1]: Comparison of the serum level of sE-selectin between the participants with and without ocular problems within each group(ANOVA, Tukey post hoc).
p-value[2]: Comparison of the serum level of sE-selectin between the SM exposed and the control groups (ANOVA, Tukey post hoc).
Bold data shows significant differences with p value <0.05.

integrin and basal cells that are the primary targets of mustard gas [25]. Abe et al. [26] described that in acute phase of SM injury, endothelial level of ICAM showed about 50% decrease from normal but gradually returned to normal level during the healing process. This finding suggested that special role of ocular microenvironment in determining the lymphocyte localization in inflammatory conditions as a normal immune response [26–28]. Such a response may decrease under the immunosuppressive conditions like in SM-exposed patients [14] which reported that serum levels of sL-selectin and sP-selectin but not E selectin decreased in SM-exposed subjects compared to the matched controls. P-selectin plays an important role in initial phase of ocular inflammation and E-selectin contributes to continued cellular infiltration at the site of inflammation [29] which is in parallel with the results of this study.

It is well defined that selectins, chemokines, integrins and other adhesion molecules are involved in the process of leucocyte homing to specific inflammatory sites in response to inflammatory stimuli [30]. Hematopoeitic stem cells (HSC) are also known to be recruited from the blood vessels into the surrounding tissues in a process largely reliant on similar processes to that of the leucocytes [31]. In addition to leucocyte and HSCs, selective recruitment of mesenchymal stem cells (MSCs) to damaged tissues is an important process in tissue repair [31]. E- and P-selectins are involved in stem cells homing and tissue repair and it seems that the change is a regulatory mechanism for inhibition of SM induced ocular problems. However, the local level of these factors is important and further investigations are required in SM exposed individuals with severe ocular problems.

Declaration of interest

The authors report no conflict of interest.

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