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Alteration in serum levels of ICAM-1 and P-, E- and L-selectins in seriously eye-injured long-term following sulfur-mustard exposure

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ABSTRACT

Introduction: In this study, the serum levels of soluble intercellular adhesion molecule 1 (ICAM-1), P-, E-, and L-selectins were investigated in seriously eye-injured patients exposed to sulfur mustard (SM).

Material and methods: A total of 128 individuals with SM-induced serious eye injuries and 31 healthy male controls were included in this study. The serum concentration of soluble forms of adhesion molecules was measured by enzyme-linked immunosorbent assay (ELISA) method.

Result: The serum level of soluble ICAM-1 was significantly higher in the SM-exposed individuals with an abnormality in tear meniscus height, corneal verticillata, and pannus compared with SM-exposed individuals without these abnormalities. There were no significant differences in the level of all three measured selectins between the SM-exposed group and the control groups. SM-exposed individuals with corneal defect had a significantly higher level of soluble E-selectin than SM-exposed individuals without this abnormality. The serum level of soluble P-selectin in the SM-exposed group with limbal abnormality was significantly lower than that in the SM-exposed without this abnormality; also it was significantly higher in SM-exposed group with fundus abnormality compared to that in the control group or SM-exposed group without this abnormality.

Conclusion: The changes in the levels of selectins and ICAM-1 in the SM-exposed group with various ocular abnormalities is a defense mechanism against the toxicity of SM. Further analysis is required to understand the molecular mechanisms of the relationship between adhesion molecules with ocular complications in SM-exposed individuals.

1. Introduction

Cell adhesion molecules are glycoproteins that mediate cell-to-cell adhesion. They consist of three main families; selectins, immunoglobulin superfamily, and integrins. Selectins include platelet activation-dependent granule-external membrane protein (P-selectin),

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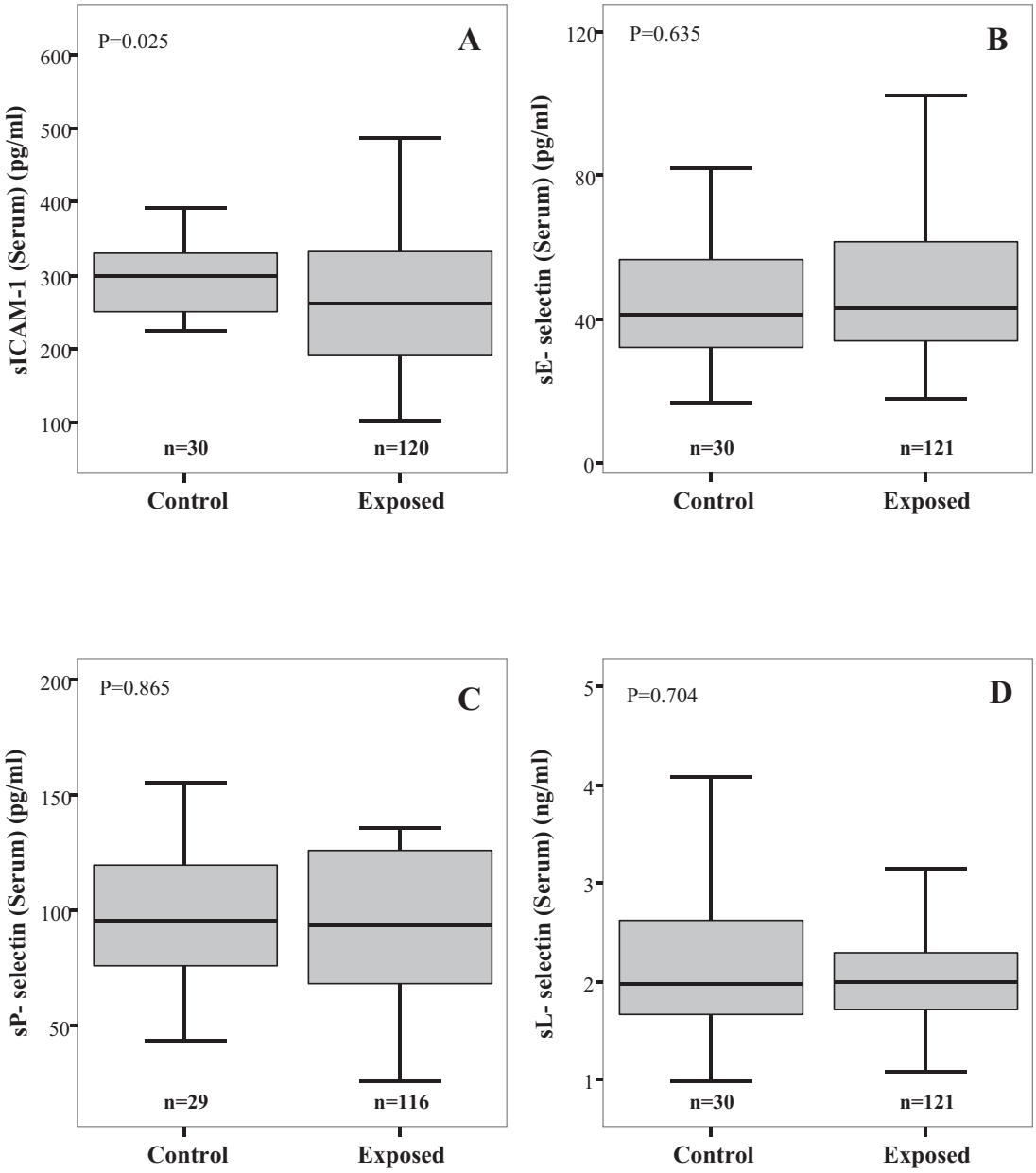

Fig. 1. Comparisons of the serum levels of adhesion molecules (pg/ml) between SM-exposed and control groups. A: sICAM-1, B: sE-selectin, C: sP-selectin and D: sL-selectin. Data represented as Median (first and third quartiles).

endothelial leukocyte adhesion molecule (E-selectin), and leukocyte-endothelial cell adhesion molecule-1 (L-selectin). They are involved in leukocytes attachment to vascular endothelium [1–3]. P-selectin is produced and stored in the alpha-granules of the platelets and Weibel–Palade bodies of endothelial cells. In activated endothelial cells and platelets, P-selectin is translocated to the cell membrane and mediates the rolling of polymorphonuclear cells on endothelial cells. E-selectin is not expressed on resting vascular endothelial cells but is rapidly induced by interleukin (IL)-1 and tumor necrosis factor (TNF). L-selectin is expressed on most leukocytes and mediates lymphocytes binding to high endothelial venules of lymph nodes and leukocyte interactions with vascular endothelial cells at sites of inflammation as a rolling receptor [4]. Members of the immunoglobulin superfamily include the intercellular adhesion molecule 1 (ICAM-1), ICAM-2 and vascular cell adhesion molecule 1 (VCAM-1). ICAM-1 is continually expressed in low concentrations on a variety of cell membranes such as leukocytes and endothelial cells. ICAM-1 is required for neutrophil migration into the sites of inflammation, and its production can be markedly increased by inflammatory stimuli [5].

The soluble form of adhesion molecules (P-selectin, E-selectin, L-selectin, and ICAM-l) can also be found in human plasma or serum as circulating proteins [6–8]. Increased levels of the soluble form of adhesion molecules have been reported in a variety of inflammatory diseases such as arteriosclerosis, vasculitis, arthritis, renal and hepatic diseases, ischemia-reperfusion conditions, organ rejection, metastasis, and many more pathologic conditions [9–11]. The association between cell adhesion molecules with the development of ocular inflammatory disorders such as uveitis, herpetic keratitis, and corneal allograft rejection has been confirmed [12]. ICAM-1 is expressed on corneal epithelial cells with chronic allograft rejection, herpetic stromal keratitis, zoster keratitis, chemical burns, atopic keratitis, fungal keratitis, and bacterial keratitis. E-selectin is expressed on the endothelial cells of limbal vessels and endothelial cells of vessels in the stroma of corneas with chronic inflammatory diseases [13]. It has been shown that E-

Table 1
Association of adhesion molecules with severity of ocular injury (Mild, Moderate and severe) in Sulfur mustard group and their comparison with control group.

	Ophthalmic complication severity	N	Median	Q_1	Q_3	Mean	SD	P-value[1]	P-value[2]	P-value[3]
sICAM-1 (serum) (pg/mL)	Control	30	14.98	12.52	16.47	15.681	4.678			
	Mild	18	12.685	9.501	19.2	15.031	8.592	0.317		
	Moderate	28	12.115	9.485	16.015	12.844	4.374	**0.033**	0.485	
	Severe	74	13.235	10.16	16.4	13.656	5.394	**0.035**	0.832	0.639
sE- selectin (serum) (pg/mL)	Control	30	4.109	3.232	5.641	4.555	1.866			
	Mild	18	3.632	3.129	6.234	4.389	1.963	0.655		
	Moderate	28	4.021	3.3	4.99	4.485	1.866	0.926	0.702	
	Severe	75	4.442	3.443	6.348	4.932	1.880	0.366	0.206	0.258
sL- selectin (serum) (pg/mL)	Control	30	19.81	16.59	26.28	22.020	7.617			
	Mild	18	19.81	17.25	22.97	20.329	4.545	0.815		
	Moderate	28	18.985	16.88	21.175	19.924	5.967	0.401	0.500	
	Severe	75	20.73	17.15	23.67	21.368	6.729	0.907	0.793	0.281
sP- selectin (serum) (pg/mL)	Control	29	4.8	3.8	6	6.052	4.398			
	Mild	17	4.4	3.2	5.2	4.353	1.301	0.255		
	Moderate	28	4.9	3.95	6.55	7.618	6.171	0.587	0.137	
	Severe	71	4.7	3.4	6.4	6.804	5.699	0.888	0.308	0.470

SM-exposed group was categorized in three sub groups (mild/moderate/severe) according to present ocular problems. Adhesion molecules level was compared between all subgroups with control and each other via Mann-Whitney test.
P-value[1]: Comparison of SM-exposed subgroups (mild, moderate, and severe) with control group.
P-value[2]: Comparison of moderate and severe subgroups with mild group.
P-value[3]: Comparison of moderate with sever subgroups.
P-values < 0.05 are in bold.
Abbreviations: N: Number; Q1 and Q3: first, and third quartiles; SD: standard deviation; pg/mL: picograms per millilitre; sICAM-1: soluble intercellular adhesion molecule 1; sE- Selectin: soluble E- Selectin; sL- Selectin: soluble L- Selectin; sP- Selectin: soluble P- Selectin.

selectin and ICAM-1 are upregulated in the conjunctiva of patients with the ocular allergic disease [14] and Mooren's ulcer [15]. Soluble E-selectin induces angiogenesis in the rat cornea and stimulates chemotaxis and tube formation of human dermal microvascular endothelial cells (HDMEC) [16,17]. P-selection has a functional role in the recruitment of eosinophils to the cornea [18]. It has been shown that L-selectin is involved in the inflammatory process during the development and progression of proliferative vitreoretinopathy (PVR) [19].

Eyes are very sensitive to SM. We have shown that photophobia, ocular surface discomfort (burning, itching, and redness), bulbar conjunctiva, and limbal tissue abnormalities are the most significant signs and symptoms in SM-exposed population 20 years after exposure in the context of Sardasht-Iran Cohort Study (SICS) [20]. Some effects of SM on the immune system have been described previously [21], and alteration of serum soluble selectins have also been reported [20]. In this study, the changes in serum ICAM-1 and plasma soluble P-, E-, L-selectins levels in SM-exposed male veterans with serious eye injuries were assessed and compared with healthy people. It should be noted that we have previously evaluated the relationship between serum ICAM-1, P-, E-, and L-selectins levels with eye injuries in a group of patients with mild SM-induced ocular problems in the context of SICS [21]. But in this study, the same associations are investigated in a group of patients with seriously SM-induced eye injuries regardless of the geographic location of exposure.

2. Materials and methods

2.1. Study design and participants

This study was performed on 128 male veterans with SM-induced serious eye injuries and 31 healthy male volunteers from the patients' family members with the same age who were gathered in a curative meeting.

2.2. Ethical considerations

Ethical approval was given from the Ethics Committee of Board of Research Ethics of Janbazan Medical and Engineering Research Center (JMERC), the Board of Research of the Ministry of Health and Medical Education, and Shahed University. All subjects provided written informed consent before entering the study.

2.3. Clinical evaluation

A case history was obtained for the primary ocular manifestations such as photophobia, ocular surface discomfort (burning, itching, and redness), foreign-body sensation, tearing, pain, blurring of vision, and dry eye sensation. Then using a slit lamp biomicroscope (NIDEK, Japan), a complete ocular examination was performed, including detecting abnormalities in lid (meibomian gland dysfunction [MGD], trichiasis, anterior blepharitis and punctal abnormalities), tear status (tear break up time [TBUT] test and tear meniscus height measurement), bulbar conjunctiva (limbal ischemia, hyperemia, abnormal vessels, pterygium, and subconjunctival fibrosis), limbal tissue (pigmentation, abnormal vessels, and pannus), cornea (calcium deposition, melting, verticillata, vascularization, epithelial, stromal, and endothelial abnormalities), lens (nuclear sclerotic [NS], cortical [C] and posterior subcapsular [PS] cataract) and fundus. The posterior segment examination conducted by using direct and indirect ophthalmoscope (Heine Omega 100 EN20-1 Binocular Indirect Ophthalmoscope Germany). History of any ocular surgery was recorded, too.

Based on the severity of the disease, the patients were classified in three groups (mild, moderate, and severe) according to the chart of the Foundation of Martyrs and Veterans Affairs (Iranian Ophthalmic Committee of Chemical Warfare Veterans) [22]. Demographic and clinical data of the study group were described in our previous study [23].

2.4. Serum preparation

Blood samples of all participants were drawn into the Vacutainer tubes (BD Biosciences) and used to prepare the serum. After the formation of the serum following clotting, the serum was removed, aliquoted, and stored for further use at temperature $-80\,^{\circ}C$.

2.5. Adhesion molecules measurement

These four adhesion molecules were selected because they represent

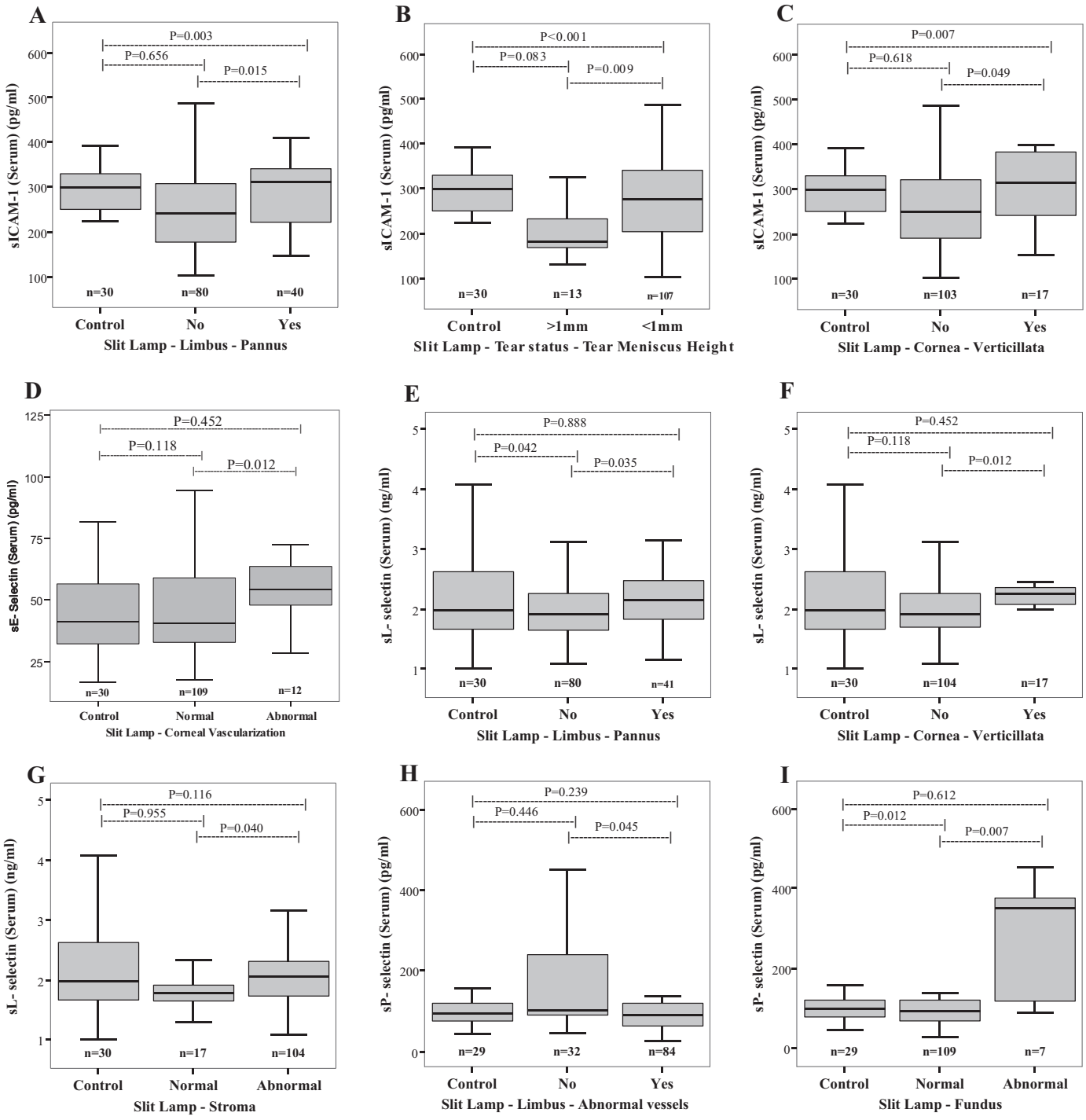

Fig. 2. Comparisons of the serum levels of adhesion molecules (pg/ml) between SM-exposed with different ocular disorders and control groups (pairwise comparisons were done using the Wilcoxon-Mann-Whitney test).

markers implicated in infectious disease pathobiology, including inflammation and endothelial activation. The serum concentration of adhesion molecules (soluble form of ICAM-1, E-selectin, L-selectin, and P-selectin) in SM-exposed and control groups was measured using Human DuoSet® ELISA Development kits (R&D Systems, USA) according to the manufacturers' instructions as described previously [24]. All ELISA kits were verified before use, and by dilutional curve testing for serum samples of participants, for each biomarker, appropriate sample dilutions were obtained. Adhesion molecules concentrations (pg/mL) were extrapolated from the corresponding standard curve.

2.6. Statistical analysis

The collected data are presented as mean (SD) and median (the first and third quartiles). The statistical comparison of adhesion molecules between the SM-exposed and control groups was performed using the Mann-Whitney nonparametric test. Also, this test was performed for the comparison between different SM-exposed groups. A difference with $P \leq 0.05$ was considered as statistically significant. Analyses of the data were performed in SPSS 23 (IBM Co., Armonk, USA).

Table 2
Association of serum level of sICAM-1 with ocular findings in Sulfur mustard group and their comparison with control group.

Slit lamp		sICAM-1 (serum) (pg/mL)						P-value[1]	P-value[2]
		N	Median	Q_1	Q_3	Mean	SD		
	Control	30	14.98	12.52	16.47	15.681	4.678		
Tear status - tear meniscus height	> 1 mm	13	9.06	8.422	11.6	10.302	3.167	**0.000**	
	< 1 mm	107	13.84	10.13	17.17	14.082	5.878	0.083	**0.009**
Tear status – TBUT	> 10 "	19	11.520	8.638	14.890	11.844	3.902	**0.004**	
	< 10 "	101	13.290	9.892	16.960	14.017	5.999	0.063	0.136
Bulbar conjectiva - subconjectival fibrosis	No	71	12.03	9.448	15.45	12.785	4.299	**0.003**	
	Yes	49	14.73	9.892	17.43	14.959	7.241	0.405	0.119
Limbus - abnormal vessels	No	33	11.78	7.729	15.99	13.607	7.804	**0.033**	
	Yes	87	13.27	10.15	16.93	13.698	4.821	**0.044**	0.290
Limbus – pannus	No	80	12.055	8.808	15.34	12.917	5.680	**0.003**	
	Yes	40	15.57	11.065	17.065	15.184	5.689	0.656	**0.015**
Cornea - verticillata	No	103	12.47	9.501	16.05	13.316	5.670	**0.007**	
	Yes	17	15.74	12.03	19.2	15.836	6.000	0.618	**0.049**
Stroma	Normal	17	11.52	8.422	15.01	11.836	4.682	**0.015**	
	Abnormal	103	13.61	9.754	16.78	13.976	5.883	**0.048**	0.172
Corneal vascularization	Normal	108	13.235	9.511	16.855	13.824	5.918	**0.041**	
	Abnormal	12	11.17	9.834	15.37	12.309	4.002	**0.022**	0.473
Fundus	Normal	113	12.53	9.521	16.29	13.540	5.851	**0.013**	
	Abnormal	7	16.93	13.2	18.09	15.823	3.639	0.415	0.108

Adhesion molecule level was compared between all slit lamp ocular findings (no/yes or normal/abnormal) subgroups with control and each other via Mann-Whitney test.
P-value[1]: Comparison of SM-exposed subgroups with control group.
P-value[2]: In the SM-exposed group comparison between with and without ocular findings.
P-values $<$ 0.05 are in bold.
Abbreviations: N: Number; Q1 and Q3: first, and third quartiles; SD: standard deviation; pg/mL: picograms per millilitre; sICAM-1: soluble intercellular adhesion molecule 1; TBUT: tear breakup time.

Table 3
Association of serum level of sE-Selectin with ocular findings in Sulfur mustard group and their comparison with control group.

Slit lamp		sE- selectin (serum) (pg/mL)						P-value[1]	P-value[2]
		N	Median	Q_1	Q_3	Mean	SD		
	Control	30	4.109	3.232	5.641	4.555	1.866		
Tear status - tear meniscus height	> 1 mm	13	4.32	3.238	4.655	4.055	0.874	0.781	
	< 1 mm	108	4.309	3.379	6.283	4.831	1.962	0.567	0.349
Tear status – TBUT	> 10 "	19	3.975	3.238	5.336	4.327	1.521	0.853	
	< 10 "	102	4.374	3.379	6.234	4.826	1.945	0.552	0.454
Bulbar conjectiva - subconjectival fibrosis	No	71	4.32	3.301	6.252	4.758	1.828	0.613	
	Yes	50	4.264	3.443	5.365	4.733	1.989	0.747	0.943
Limbus - abnormal vessels	No	33	4.179	3.483	4.888	4.337	1.417	0.956	
	Yes	88	4.381	3.23	6.411	4.902	2.023	0.518	0.425
Limbus – pannus	No	80	4.055	3.39	6.082	4.673	1.910	0.867	
	Yes	41	4.58	3.378	6.313	4.894	1.860	0.379	0.488
Cornea - verticillata	No	104	4.374	3.374	6.202	4.762	1.910	0.614	
	Yes	17	3.941	3.409	5.431	4.661	1.803	0.894	0.858
Stroma	Normal	17	3.745	3.191	4.715	3.903	1.110	0.268	
	Abnormal	104	4.429	3.394	6.315	4.886	1.956	0.422	0.066
Corneal vascularization	Normal	109	4.067	3.301	5.903	4.665	1.931	0.888	
	Abnormal	12	5.45	4.81	6.363	5.499	1.266	**0.042**	**0.035**
Fundus	Normal	114	4.334	3.37	6.169	4.729	1.836	0.625	
	Abnormal	7	3.845	3.631	6.473	5.057	2.771	0.969	0.912

Adhesion molecule level was compared between all slit lamp ocular findings (no/yes or normal/abnormal) subgroups with control and each other via Mann-Whitney test.
P-value[1]: Comparison of SM-exposed subgroups with control group.
P-value[2]: In the SM-exposed group comparison between with and without ocular findings.
P-values $<$ 0.05 are in bold.
Abbreviations: N: Number; Q1 and Q3: first, and third quartiles; SD: standard deviation; pg/mL: picograms per millilitre; sE- Selectin: soluble E- Selectin; TBUT: tear breakup time.

3. Results

3.1. Clinical findings

In the previous article [23], patients' information, including ocular history, demographic information, slit-lamp examination results, and ophthalmoscopic findings were described. In short, the mean age of the veterans and the control group was 44.86 and 50 years, the percentage of disability among veterans was 58.85% based on the chart of the Foundation of Martyrs and Veterans Affairs, and the mean period of

Table 4
Association of serum level of sL-Selectin with ocular findings in Sulfur mustard group and their comparison with control group.

Slit lamp		sL- selectin (serum) (pg/mL)						P-value[1]	P-value[2]
		N	Median	Q_1	Q_3	Mean	SD		
	Control	30	19.81	16.59	26.28	22.020	7.617		
Tear status - tear meniscus height	> 1 mm	13	17.45	15.08	22.19	20.262	7.700	0.328	
	< 1 mm	108	20.1	17.27	23.18	20.954	6.110	0.818	0.295
Tear status – TBUT	> 10 "	19	20.46	17.24	23.33	22.088	6.941	0.853	
	< 10 "	102	19.95	17.09	22.97	20.654	6.143	0.623	0.540
Bulbar conjectiva - subconjectival fibrosis	No	71	19.21	16.37	22.6	20.093	5.993	0.370	
	Yes	50	20.49	17.58	23.52	21.996	6.533	0.698	0.116
Limbus - abnormal vessels	No	33	20.79	17.27	23.2	21.352	6.209	0.978	
	Yes	88	19.905	17.085	22.83	20.702	6.314	0.606	0.547
Limbus – pannus	No	80	19.11	16.455	22.575	19.906	5.548	0.319	
	Yes	41	21.53	18.24	24.74	22.777	7.171	0.435	**0.024**
Cornea - verticillata	No	104	19.11	16.94	22.66	20.510	6.448	0.452	
	Yes	17	22.55	20.73	23.67	23.139	4.533	0.188	**0.012**
Stroma	Normal	17	17.88	16.42	19.21	18.344	4.859	0.116	
	Abnormal	104	20.625	17.28	23.18	21.294	6.391	0.955	**0.040**
Corneal vascularization	Normal	109	20.14	17.24	22.97	20.859	6.132	0.720	
	Abnormal	12	18.785	17.255	22.2	21.063	7.692	0.749	0.687
Fundus	Normal	114	20.1	17.25	23.16	20.999	6.331	0.781	
	Abnormal	7	18.24	15.11	20.73	18.931	5.076	0.332	0.287

Adhesion molecule level was compared between all slit lamp ocular findings (no/yes or normal/abnormal) subgroups with control and each other via Mann-Whitney test.
P-value[1]: Comparison of SM-exposed subgroups with control group.
P-value[2]: In the SM-exposed group comparison between with and without ocular findings.
P-values $<$ 0.05 are in bold.
Abbreviations: N: Number; Q1 and Q3: first, and third quartiles; SD: standard deviation; pg/mL: picograms per millilitre; sL- Selectin: soluble L- Selectin; TBUT: tear breakup time.

Table 5
Association of serum level of sP-Selectin with ocular findings in Sulfur mustard group and their comparison with control group.

Slit lamp		sP- selectin (serum) (pg/mL)						P-value[1]	P-value[2]
		N	Median	Q_1	Q_3	Mean	SD		
	Control	29	4.8	3.8	6	6.052	4.398		
Tear status - tear meniscus height	> 1 mm	12	5.05	3.2	12.05	7.508	6.264	0.977	
	< 1 mm	104	4.7	3.5	6.2	6.541	5.402	0.847	0.895
Tear status – TBUT	> 10 "	19	5.2	3.4	6.5	6.158	4.331	0.704	
	< 10 "	97	4.6	3.4	5.9	6.736	5.686	0.761	0.511
Bulbar conjectiva - subconjectival fibrosis	No	66	4.9	3.6	6	6.268	4.944	0.971	
	Yes	50	4.45	3.2	6.5	7.134	6.122	0.760	0.776
Limbus - abnormal vessels	No	32	5.15	4.45	12	8.331	6.628	0.239	
	Yes	84	4.45	3.2	6	5.998	4.858	0.446	**0.045**
Limbus – pannus	No	76	4.7	3.4	6	6.689	5.666	0.807	
	Yes	40	4.8	3.8	6.6	6.550	5.160	0.995	0.809
Cornea - verticillata	No	102	4.7	3.4	6.1	6.504	5.390	0.752	
	Yes	14	5.1	3.8	6.6	7.643	6.181	0.560	0.448
Stroma	Normal	15	5.4	3.4	6.5	6.993	5.904	0.560	
	Abnormal	101	4.6	3.4	6.1	6.589	5.438	0.746	0.348
Corneal vascularization	Normal	105	4.7	3.4	6.3	6.810	5.719	0.797	
	Abnormal	11	5.1	3.9	6.3	5.036	1.180	0.694	0.634
Fundus	Normal	109	4.6	3.4	6	6.208	5.046	0.612	
	Abnormal	7	17.5	5.1	19.4	13.386	7.729	0.012	**0.007**

Adhesion molecule level was compared between all slit lamp ocular findings (no/yes or normal/abnormal) subgroups with control and each other via Mann-Whitney test.
P-value[1]: Comparison of SM-exposed subgroups with control group.
P-value[2]: In the SM-exposed group comparison between with and without ocular findings.
P-values $<$ 0.05 are in bold.
Abbreviations: N: Number; Q1 and Q3: first, and third quartiles; SD: standard deviation; pg/mL: picograms per millilitre; sP- Selectin: soluble P- Selectin; TBUT: tear breakup time.

illness was 21.58 years. With regards to the exposure with SM, 65.2% of the patients exposed once, 24.3% twice, and 10.5% more than twice. The minimum numbers of the symptoms were 3 to 5 in the majority of SM-exposed patients. The most commonly reported ocular symptoms were photophobia with 73.2%, blurred vision with 72.5%, dry eye sensation with 66.4%, a sensation of foreign body with 61.1%, tearing with 46.3%, and pain with 43%. Summary of the frequency of various ocular abnormalities and the severity of ocular damage based on slip lamp examination in SM-exposed patients are summarized in the Supplementary Table.

3.2. Serum levels of soluble adhesion molecules

Comparisons of the serum levels of soluble adhesion molecules (ICAM-1, E-selectin, L-selectin, and P-selectin) in SM-exposed and control groups are shown in Fig. 1. As indicated in Fig. 1A, the serum level of soluble ICAM-1 was significantly low in the SM-exposed group compared to the control group. There were no significant differences in the level of all three measured selectins between the SM-exposed and control groups (Fig. 1B, C, and D).

3.3. Association of adhesion molecules with the severity of the ocular injury

Regarding the severity of the ocular injury, we observed significantly lower levels of soluble ICAM-1 in the exposed group with moderate and severe ocular involvement compared to the control group (Table 1). The observed differences between SM-exposed individuals with mild, moderate, or severe ocular injuries with the control were not significant (Table 1).

3.4. The association between serum level of soluble adhesion molecules and slit lamp findings in SM-exposed group

The serum level of soluble ICAM-1 in the SM-exposed individuals with abnormalities in tear meniscus height, pannus, and verticillata was significantly higher than that in the SM-exposed individuals without these complications (Fig. 2 and Table 2). SM-exposed individuals with corneal vascularization had a significantly higher level of soluble E-selectin than that in SM-exposed persons without this abnormality (Fig. 2D and Table 3). Comparisons of the levels of soluble L-selectin between SM-exposed individuals with different ocular disorders and control groups are presented in Table 4. The significantly higher level of soluble L-selectin was identified in SM-exposed group with corneal verticillata, and stromal abnormalities (Fig. 2F and G) and limbal abnormality (pannus) (Fig. 2E) in comparison to the SM-exposed group without these abnormalities. Also, soluble L-selectin in SM-exposed individuals with normal limbus was significantly lower than that in the control group (Fig. 2E). Table 5 presents the results of soluble P-selectin measurement in association with various ocular abnormalities among veterans. As it is seen in Fig. 2H, the serum level of soluble P-selectin in the SM-exposed group with limbal tissue abnormality (abnormal vessels, 8.3 pg/mL), was significantly lower than that in the SM-exposed without this abnormality (5.9 pg/mL). SM-exposed individuals with abnormal fundus had a significantly higher level of soluble P-selectin compared to the SM-exposed group without this abnormality (Fig. 2I, $P = 0.007$).

4. Discussion

In this study, the association between serum soluble adhesion molecules, including ICAM-1, E-selectin, L-selectin, and P-selectin with ocular complications was investigated in SM-exposed individuals with eye injuries regardless of their geographical distribution. The findings of this study showed that the serum level of soluble ICAM-1 was significantly lower in SM-exposed individuals with ocular problems. Further analysis showed that serum soluble ICAM-1 level in the SM-exposed group with normal tear meniscus height, normal tear break up time (TBUT), without subconjunctival fibrosis, with and without abnormal limbal vessels, with no pannus and corneal verticillata, with and without stromal abnormality and corneal vascularization, as well as with normal fundus had significantly lower serum levels of sICAM-1 compared to control group. The elevated soluble ICAM-1 level was also observed in residents of Sardasht City, Iran exposed to SM with blurred vision compared to SM-exposed without this abnormality, yet overall the difference in ICAM-1 level was insignificant between SICS groups. Also, almost none of ocular signs and symptoms had significantly different serum level of sICAM-1 compare to control group in SICS which eye complications were mild toxicity, except for blurred vison as noted above [24]. It seems that the serum level of soluble ICAM-1 is elevated in eye abnormalities induced by SM-exposure. Increased level of soluble ICAM-1 in the serum of patients with intermediate uveitis has been reported, and it was significantly related to intraocular inflammation [25]. It should be noted that the serum level of soluble ICAM-1 was significantly lower in SM-exposed subjects with moderate and severe ocular injuries compare to control group, yet subgroup with mild ocular injury had insignificant difference with controls as SICS. This finding suggests the possibility of introducing soluble ICAM-1 as a marker of ocular diseases in SM-exposed populations, which requires further analysis.

Analysis of the serum levels of soluble selectins, including P-, E-, and L-selectin, showed no significant differences between the SM-exposed group with ocular abnormalities and the control group. In the context of SICS, alterations in the serum levels of soluble P-, E- and L-selectins were observed 20 years after sulfur mustard exposure. Soluble P-selectin and soluble L-selectin were significantly lower in the SM-exposed group compared to the control group, but soluble E-selectin was significantly higher in the sera of the SM-exposed group compared to the control group [26]. Also, almost none of ocular signs and symptoms had significantly different serum level of sL-selectin compare to control group in SICS [24]. In current study, none of ocular signs and symptoms had significantly different level of sL-selectin compared with the control group as SICS. However, in SICS, serum level of sP-selectin was significantly lower and sE-selectin higher than the control group in SM-exposed subjects with no ocular signs and symptoms (R25: PMID: 23370300). In the current study, only significantly higher level of sE-selectin was noted in SM-exposed subjects with corneal vascularization compare to control group, yet none of ocular signs and symptoms had a significantly different level of sP-selectin compared with the control group in contrast to SICS. It seems that soluble E-selectin has a role in immunological responses in SM-induced lesions. But findings from this study regarding the association between soluble P-, E, and L-selectins with the SM-exposure were not in agreement with the results of SICS, as there was an insignificant difference in all three types of selectins between two study groups in the current study. This discrepancy may be due to the severity of intoxication or environmental and geographical differences. Almost all of the participants of this study have suffered from severe SM-induced eye injuries; however, a few SM-exposed participants of SICS have suffered from serious eye injuries. Contrary to the SICS, the samples in this study were not limited to a particular geographic area of Iran.

Concerning the association between serum soluble selectins levels and ocular abnormalities, the results revealed a high level of soluble E-selectin in SM-exposed individuals with corneal vascularization, high level of soluble L-selectin in SM-exposed with limbal abnormality (pannus), corneal verticillata, and stromal abnormality, high level of soluble P-selectin in SM-exposed with abnormal fundus, and low level of soluble P-selectin in SM-exposed with limbal tissue abnormality (abnormal vessels), compared to SM-exposed groups without these abnormalities. In SICS, soluble P-selectin level in SM-exposed group with limbal abnormality was significantly higher than that in the SM-exposed group without limbal abnormality, while serum level of soluble P-selectin in the SM-exposed group without any ocular problems was significantly lower than that in the control group without any ocular problems. Serum soluble E-selectin level in SM-exposed group with photophobia was significantly higher than that in the SM-exposed with normal ocular conditions. Serum level of soluble L-selectin in the SM-exposed group with photophobia was significantly higher than that in the SM-exposed group without this abnormality [24]. Therefore, a high level of soluble E-selectin in SM-exposed with corneal vascularization and photophobia may clinically indicate the possible progression of the disease to corneal involvement.

An increased concentration of circulating soluble E-selectin has been demonstrated in numerous human diseases, including rheumatoid

arthritis, juvenile idiopathic arthritis, breast cancer, gastric cancer, Crohn's disease, asthma, sepsis, and acute respiratory disease syndrome. Harrington et al. have shown that a high level of circulating E-selectin in serum may be due to endothelial cell apoptosis [27]. Thus, it seems that the high level of soluble E-selectin in SM-exposed individuals with corneal vascularization is caused by vascular endothelial cell apoptosis. Because of partially inhibitory effects of soluble E-selectin on leukocyte adhesion to activated endothelial cells (unlike E-selectin), leukocyte mobility and endothelial cells activity may be suppressed in these patients [28].

The increased level of soluble L-selectin in SM-exposed individuals with limbal abnormality (pannus) or corneal verticillata and stromal abnormality may also suppress parts of the immune responses. Although, the presence of a soluble form of L-selectin in the serum may reflect L-selectin expression on most of the peripheral blood leukocytes, relatively high concentration of soluble L-selectin can decrease L-selectin-mediated leukocyte adhesion [21,29].

Plasma soluble P-selectin levels might represent a useful marker of in vivo platelet activation [30]. In this study, soluble P-selectin levels in SM-exposed individuals with limbal tissue abnormality was low, but in SM-exposed individuals with abnormal fundus, it was high in comparison to SM-exposed group without these abnormalities. Alterations in the serum levels of soluble P-selectin in the SM-exposed group may result from changes in total expression of P-selectin and number of activated platelets. The important point to mention as the limitation of this study is that as it is an observational study and the time between SM exposure and analysis is > 20 years. Hence, any findings are associative and may not allow inferring causality.

5. Conclusions

In conclusion, the change in selectins and ICAM-1 levels in the SM-exposed group with various ocular abnormalities could be a defense mechanism against the toxicity of SM, although this possibility is not definite due to the study design and the data provided. Generally, serum level of ICAM-1 was lower in SM-exposed subjects. However, further studies such as the assessments of the local level of adhesion molecules are required to determine the association between ocular abnormalities in SM-exposed people with these factors.

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List of abbreviations

SM	sulfur mustard
ICAM-1	intercellular adhesion molecule 1
VCAM-1	vascular cell adhesion molecule 1
IL-1	interleukin 1
ARDS	acute respiratory disease syndrome
JMERC	Janbazan Medical and Engineering Research Center
HDMEC	Human dermal microvascular endothelial cells
MGD	Meibomian gland dysfunction
TBUT	tear break up time
NS	nuclear sclerotic
C	cortical
PS	posterior subcapsular

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Declaration of competing interest

The authors report no conflict of interest in this study.

References

[1] S.D. Rosen, C.R. Bertozzi, The selectins and their ligands, Curr. Opin. Cell Biol. 6 (5) (1994) 663–673 (Epub 1994/10/01).
[2] N. Hogg, Roll, roll, roll your leucocyte gently down the vein, Immunol. Today 13 (4) (1992) 113–115 (Epub 1992/04/01).
[3] L.A. Lasky, Lectin cell adhesion molecules (LEC-CAMs): a new family of cell adhesion proteins involved with inflammation, J. Cell. Biochem. 45 (2) (1991) 139–146 (Epub 1991/02/01).
[4] K. Ley, The role of selectins in inflammation and disease, Trends Mol. Med. 9 (6) (2003) 263–268 (Epub 2003/06/28).
[5] M.L. Dustin, R. Rothlein, A.K. Bhan, C.A. Dinarello, T.A. Springer, Induction by IL 1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1), J. Immunol. 137 (1) (1986) 245–254 (Epub 1986/07/01).
[6] L.C. Dunlop, M.P. Skinner, L.J. Bendall, E.J. Favaloro, P.A. Castaldi, J.J. Gorman, et al., Characterization of GMP-140 (P-selectin) as a circulating plasma protein, J. Exp. Med. 175 (4) (1992) 1147–1150 (Epub 1992/04/01).
[7] A.J. Gearing, I. Hemingway, R. Pigott, J. Hughes, A.J. Rees, S.J. Cashman, Soluble forms of vascular adhesion molecules, E-selectin, ICAM-1, and VCAM-1: pathological significance, Ann. N. Y. Acad. Sci. 667 (1992) 324–331 (Epub 1992/12/04).
[8] B. Schleiffenbaum, O. Spertini, T.F. Tedder, Soluble L-selectin is present in human plasma at high levels and retains functional activity, J. Cell Biol. 119 (1) (1992) 229–238 (Epub 1992/10/01).
[9] E. Demerath, B. Towne, J. Blangero, R.M. Siervogel, The relationship of soluble ICAM-1, VCAM-1, P-selectin and E-selectin to cardiovascular disease risk factors in healthy men and women, Ann. Hum. Biol. 28 (6) (2001) 664–678 (Epub 2001/12/01).
[10] M.P. Bevilacqua, R.M. Nelson, G. Mannori, O. Cecconi, Endothelial-leukocyte adhesion molecules in human disease, Annu. Rev. Med. 45 (1994) 361–378 (Epub 1994/01/01).
[11] A. Etzioni, Adhesion molecules–their role in health and disease, Pediatr. Res. 39 (2) (1996) 191–198 (Epub 1996/02/01).
[12] S.N. Zhu, M.R. Dana, Expression of cell adhesion molecules on limbal and neovascular endothelium in corneal inflammatory neovascularization, Invest. Ophthalmol. Vis. Sci. 40 (7) (1999) 1427–1434 (Epub 1999/06/08).
[13] W. Philipp, W. Gottinger, Leukocyte adhesion molecules in diseased corneas, Invest. Ophthalmol. Vis. Sci. 34 (8) (1993) 2449–2459 (Epub 1993/07/01).
[14] J.I. McGill, S.T. Holgate, M.K. Church, D.F. Anderson, A. Bacon, Allergic eye disease mechanisms, Br. J. Ophthalmol. 82 (10) (1998) 1203–1214 (Epub 1999/01/30).
[15] C. Kafkala, J. Choi, P. Zafirakis, S. Baltatzis, C. Livir-Rallatos, B. Rojas, et al., Mooren ulcer: an immunopathologic study, Cornea 25 (6) (2006) 667–673 (Epub 2006/11/02).
[16] A.E. Koch, M.M. Halloran, C.J. Haskell, M.R. Shah, P.J. Polverini, Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1, Nature 376 (6540) (1995) 517–519 (Epub 1995/08/10).
[17] Y. Yu, K.S. Moulton, M.K. Khan, S. Vineberg, E. Boye, V.M. Davis, et al., E-selectin is required for the antiangiogenic activity of endostatin, Proc. Natl. Acad. Sci. U. S. A. 101 (21) (2004) 8005–8010 (Epub 2004/05/19).
[18] J.T. Kaifi, L.R. Hall, C. Diaz, J. Sypek, E. Diaconu, J.H. Lass, et al., Impaired eosinophil recruitment to the cornea in P-selectin-deficient mice in Onchocerca volvulus keratitis (river blindness), Invest. Ophthalmol. Vis. Sci. 41 (12) (2000) 3856–3861 (Epub 2000/10/29).
[19] E. Toker, H. Kazokoglu, S. Sahin, Cell adhesion molecules in subretinal fluid: soluble forms of VCAM-1 (vascular cell adhesion molecule-1) and L-selectin, Int. Ophthalmol. 22 (2) (1998) 71–76 (Epub 1999/09/03).
[20] H. Ghasemi, A. Mostafaie, R. Yaraee, Z.M. Hassan, A. Rezaei, M. Mahmoudi, et al., Association of serum immunoglobulins levels and eye injuries in sulfur mustard exposed: Sardasht-Iran Cohort Study, Int. Immunopharmacol. 17 (3) (2013) 944–951 (Epub 2013/02/02).
[21] R. Yaraee, T. Ghazanfari, M. Ebtekar, S.K. Ardestani, A. Rezaei, A. Kariminia, et al., Alterations in serum levels of inflammatory cytokines (TNF, IL-1alpha, IL-1beta and IL-1Ra) 20 years after sulfur mustard exposure: Sardasht-Iran Cohort Study, Int. Immunopharmacol. 9 (13–14) (2009) 1466–1470 (Epub 2009/09/15).
[22] M. Ghassemi-Broumand, J. Aslani, S.-N. Emadi, Delayed ocular, pulmonary, and cutaneous complications of mustards in patients in the city of Sardasht, Iran, Cutan. Ocul. Toxicol. 27 (4) (2008) 295–305.
[23] H. Ghasemi, T. Ghazanfari, M. Ghassemi-Broumand, M.A. Javadi, M. Babaei, M.R. Soroush, et al., Long-term ocular consequences of sulfur mustard in seriously eye-injured war veterans, Cutan. Ocul. Toxicol. 28 (2) (2009) 71–77.
[24] H. Ghasemi, R. Yaraee, Z.M. Hassan, S. Faghihzadeh, M.R. Soroush, S. Pourfarzam, et al., Association of ophthalmic complications in patients with sulfur mustard induced mild ocular complications and serum soluble adhesion molecules: Sardasht-Iran Cohort Study, Int. Immunopharmacol. 17 (3) (2013) 980–985 (Epub 2013/02/02).
[25] A.M. Klok, L. Luyendijk, M.J. Zaal, A. Rothova, A. Kijlstra, Soluble ICAM-1 serum levels in patients with intermediate uveitis, Br. J. Ophthalmol. 83 (7) (1999) 847–851 (Epub 1999/06/25).
[26] R. Yaraee, T. Ghazanfari, S. Faghihzadeh, A. Mostafaie, M.R. Soroush, K. Inai, et al., Alterations in the serum levels of soluble L, P and E-selectin 20 years after sulfur

mustard exposure: Sardasht-Iran Cohort Study, Int. Immunopharmacol. 9 (13–14) (2009) 1477–1481 (Epub 2009/09/08).
[27] E.O. Harrington, T. Stefanec, J. Newton, S. Rounds, Release of soluble E-selectin from activated endothelial cells upon apoptosis, Lung 184 (5) (2006) 259–266 (Epub 2007/01/20).
[28] R. Pigott, L.P. Dillon, I.H. Hemingway, A.J. Gearing, Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells, Biochem. Biophys. Res. Commun. 187 (2) (1992) 584–589 (Epub 1992/09/16).
[29] T. Barkhausen, C. Krettek, M. van Griensven, L-selectin: adhesion, signalling and its importance in pathologic posttraumatic endotoxemia and non-septic inflammation, Exp. Toxicol. Pathol. 57 (1) (2005) 39–52 (Epub 2005/08/11).
[30] P. Ferroni, F. Martini, S. Riondino, F. La Farina, A. Magnapera, F. Ciatti, et al., Soluble P-selectin as a marker of in vivo platelet activation, Clin. Chim. Acta 399 (1–2) (2009) 88–91 (Epub 2008/10/07).