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ABSTRACT

Introduction: Sulfur mustard (SM) intoxication produces local and systemic changes in the human body. In this study, the relationship between tear and serum matrix metalloproteinase (MMP)-9 and serum tissue inhibitors of metalloproteinases (TIMPs) are assessed in serious eye-injured SM-exposed casualties.

Methods: A group of 128 SM-exposed patients with serious ocular injuries in three subgroups (19 mild, 31 moderate, and 78 severe cases) is compared with 31 healthy controls. Tear and ocular status and serum MMPs and MMP-9/TIMPs complex levels were evaluated using enzyme-linked immunosorbent assay (ELISA).

Results: Serum level of MMP-9 was significantly higher in the SM-exposed group compared to the control group ($P = 0.009$). Mean serum MMP-9 level in the SM-exposed group with ocular abnormalities was significantly higher than that in the SM-exposed group without ocular abnormalities. SM-exposed people with corneal calcification had significantly higher serum MMP-9/TIMP-1 level compared to the SM-exposed ones without this problem ($P = 0.045$). The SM-exposed group with severe ocular injuries had significantly higher MMP-9/TIMP-1 than the controls ($P = 0.046$). The SM-exposed group had significantly lower levels of MMP-9/TIMP-4 complex than the controls ($P < 0.001$). The SM-exposed group with tear meniscus and fundus abnormality had significantly higher MMP-9/TIMP-4 levels than the SM-exposed group without these problems ($P = 0.009$ and $P = 0.020$).

Conclusion: Serum MMP-9 level had increased in SM-exposed groups with ocular problems, while TIMP-1 and TIMP-2 levels had remained unchanged. Serum TIMP-4 drastically decreased in SM-exposed group, which clearly explains the severity of the systemic and ocular damages.

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1. Introduction

Sulfur mustard (2,2′-dichlorodiethyl sulfide; SM) ocular effects may induce by direct (local) or indirect (systemic) toxic effects. Induced damages differ in early, mid- and long-term periods [1]. The toxicity involves the immune system including matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) [2]. SM can change the expression pattern of various proteins and several proteases such as caspases and MMP and induces a wide range of abnormalities [3].

MMPs are the members of the zinc-dependent endopeptidase and are active in tissue remodeling and extracellular matrix (ECM) such as collagen, elastin, gelatin, matrix glycoproteins, and proteoglycan degradation. They are generated by connective tissues and proinflammatory cells, including fibroblasts, osteoblasts, endothelial cells, macrophages, neutrophils, and lymphocytes under control of hormones, growth factors, and cytokines. MMPs are initially expressed as zymogens and then processed to active forms by proteolytic enzymes [4]. MMPs play key roles in the normal physiologic functions of connective tissue during the process of morphogenesis and wound healing, [5]. MMPs work well in balance with four members of the tissue inhibitors of metalloproteinases (TIMPs) family for ECM deposition and maintaining. The two-domain TIMPs display many biochemical, physiological, and biological functions and inhibit activation of pro-MMP and MMPs, and prevent cell growth promotion, matrix binding, angiogenesis, and apoptosis [6]. Any imbalance in the ratio of MMP/TIMP may be associated with a tissue injury [7].

Corneal neovascularization as a delayed complication of SM exposure may lead to visual impairment. MMP and other proteolytic enzymes may contribute to the pathogenesis of this complication [8]. MMPs and TIMPs play a complex role in regulating angiogenesis. MMP-1, MMP-2, and MMP-9 dissolve ECM and initiate or promote angiogenesis, while TIMP-1, TIMP-2, TIMP-3, and possibly TIMP-4 inhibit neovascularization [9].

It has been shown that healing or ulceration of corneas are related to the proteolytic activity of MMP that can degrade all ECM components, and are involved in lots of physiological and pathological disorders in which ECM remodeling take place [2,10]. Collagenolytic activity following corneal alkali burns is characterized by a breakdown of the collagenous stromal tissue of the cornea that causes chronic ulceration [10].

One of the most important effects of SM on the cells is increasing proteinase activities that result in clinical manifestations after exposure [11]. SM-exposed rabbits with corneal impairment demonstrate a significant shift towards the positive values in stromal degrading gelatinase (MMP-2 and MMP-9) levels/activities in the aqueous humor [12]. MMPs are a multigene family with at least 26 members [13,14], of them, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) play critical roles in many pathological processes [15,16]. MMP-9 is secreted by basal corneal epithelial cells and migrates to the surface of the wound that leads to chronic corneal ulcerations. Inhibition of this enzyme activity leads to improvement of the integrity of the basement membrane [10]. Posterior portion of the cornea in contact with aqueous humor initiates the production of cytokines/chemokines, which in turn may increase MMP-2 and MMP-9 activities [17]. Mice-induced experimental dry-eye disease, increase the expression of inflammatory cytokines and MMP-9 and stimulates the signaling pathways of mitogen-activated protein kinase (MAPK) and MMPs and accelerate the pathologic cascade of dry-eye disease [18]. High-dose SM skin exposure in guinea-pigs, causes a significant increase in tissues pro and active MMP-2 and MMP-9 [19].

The small amount of tear volume restricts the measurements of tear fluid components [20]. Corneal and conjunctival tissues are linked through a thin layer of tear fluid [21]. In ocular surface disorders, tear film instability leads to corneal epithelium inflammatory damages due to goblet cell loss, inflammatory cytokine release, and MMP activation [22]. In the tear and epithelium of the conjunctiva or cornea of these patients, both types of active MMP-2 and MMP-9 are measurable, while in the normal condition, only the prototypes of MMP-2 and MMP-9 are detectable [23,24]. Tear MMP plays an active role in the pathogenesis of recurrent corneal erosions, and oral MMP inhibitors such as doxycycline have symptomatic relief in these patients [25]. Some of the relationships between serum inflammatory mediators and ocular injuries induced by SM have previously been reported [1]. In this study, the relationship between tear and serum MMP-9 and serum TIMPs levels with SM-induced ocular problems is assessed in severely eye-injured SM-exposed casualties.

2. Participants and methods

2.1. Study design and participants

In this study, a group of 128 SM exposed patients with serious ocular injuries in three subgroups (19 mild, 31 moderate, and 78 severe cases) were compared with 31 age-matched normal controls, after obtaining their written informed consents. The study design was approved by the Ethics Committee of Janbazan Medical and Engineering Research Center (JRMEC). Those participants under systemic immunosuppressive therapy or with acute infectious disorders were excluded. A comprehensive personal survey, including previous history and ocular symptoms and a complete anterior and posterior eye segments examination using slit lamp biomicroscope (NIDEK, Gamagori, Japan) and direct/indirect ophthalmoscope (Heine K180 Ophthalmoscope/Heine Omega 100 EN20-1 Binocular Indirect Ophthalmoscope Germany) was done. Lids were assessed based on the current definitions from meibomian gland dysfunction (MGD) [26,27]. Tear status was evaluated by tear break up time (TBUT) test and measuring tear meniscus height. Ocular surface, including bulbar conjunctiva, limbal tissue, cornea, lens, and anterior segment was examined. The severity of ocular involvement was graded as mild, moderate, and severe based on the War Veteran's Foundation Affairs' Chart (Iranian Ophthalmic Committee of Chemical Warfare Veterans) [28]. Detailed demographic information and obtained results of slit lamp finding were documented in our previous study [29].

2.2. Serum and tear samples collection

The serum samples were collected and separated from 2 mL venous blood of all SM-exposed and control groups after 1 h clotting at room temperature and 20 min centrifuge at 2000 $\times g$. Then the sera were aliquoted and stored at −80 °C until laboratory measurements. Tear samples were obtained from 118 exposed and 31 control volunteers in the early morning with the installation of one sterile drop (200 μL) of 5% sodium chloride solution in the inferior fornices of both eyes and gently placing sterile Weck-Cel sponges (Medtronic) in lacrimal lakes of both eyes [30]. Then, the samples were diluted with 200 μL culture medium solution of Roswell Park Memorial Institute (RPMI) in specific tubes and centrifuged at 2000 $\times g$ (for separation of the tear contents from the Weck-Cel sponges) and preserved at −80 °C.

2.3. Measuring serum concentration of MMP-9 and MMP-9/TIMPs complex

Human MMP-9, MMP-9/TIMP-1, MMP-9/TIMP-2, and MMP-9/TIMP-4 complex DuoSet® ELISA Development Kit (R&D systems, Minneapolis, USA) were used to measure tear and serum MMPs and serum MMP-9/TIMPs complex level according to the manufacturers' instructions. Briefly, microplates were pre-coated with specific primary antibodies for each MMP or MMP-9/TIMPs complex. The specific secondary antibodies were conjugated to horseradish peroxidase. The color development was stopped by acid solution (2 N sulfuric acid), and the intensity of the color was measured at 450 nm using ELISA reader

Table 1
Changes of MMP-9 and their complex according to 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]
MMP-9 (Serum) (μg/ml)	Control	29	0.485	0.281	0.754	0.553	0.379			
	Mild	18	0.567	0.306	1.028	0.747	0.601	0.336		
	Moderate	30	0.852	0.335	1.227	0.812	0.495	**0.050**	0.431	
	Severe	75	0.717	0.451	1.188	0.841	0.505	**0.005**	0.247	0.804
TIMP-1 (Serum) (μg/ml)	Control	31	10.615	3.972	14.390	13.483	14.446			
	Mild	19	11.460	4.251	22.385	20.574	33.672	0.516		
	Moderate	30	13.618	3.812	22.915	18.309	20.830	0.445	0.902	
	Severe	78	15.743	4.651	23.350	21.910	27.653	**0.046**	0.304	0.313
TIMP-2 (Serum) (μg/ml)	Control	31	0.301	0.263	0.352	0.593	1.108			
	Mild	19	0.251	0.222	0.332	0.260	0.070	**0.016**		
	Moderate	31	0.296	0.205	0.372	0.348	0.300	0.269	0.412	
	Severe	78	0.292	0.222	0.329	0.625	1.823	0.132	0.361	0.770
TIMP-4 (Serum) (ng/ml)	Control	30	43.405	26.100	61.820	73.275	102.326			
	Mild	19	24.650	16.630	27.980	168.794	623.011	**0.002**		
	Moderate	31	24.870	17.410	35.700	35.257	44.512	**0.001**	0.555	
	Severe	78	25.180	16.920	33.580	78.693	311.132	**0.000**	0.601	0.799
MMP-9 (Tear) (μg/ml)	Control	21	0.913	0.087	2.108	1.002	0.945			
	Mild	18	0.305	0.070	0.787	0.597	0.783	0.215		
	Moderate	30	0.227	0.038	1.613	0.787	0.992	0.293	0.975	
	Severe	74	0.254	0.085	1.053	0.744	0.968	0.337	0.687	0.568

SM-exposed group was categorized in there sub groups (mild/moderate/severe) according to present ocular problems. MMP-9 and their complex 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.
MMP: Matrix metalloproteinase.
TIMP: Tissue Inhibitor of Metalloproteinase.
SM: Sulfur mustard.

(Stat-Fax 2600).

2.4. Statistical analysis

Statistical analyses were performed in SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Findings are expressed as Mean ± SD or median (minimum-maximum). Comparison of MMP-9 and MMP-9/TIMPs complex among studied groups were performed by Mann-Whitney test and interquartile range. Because of deep departure from a normal distribution, box plots were used to show the data. P values < 0.05 were considered as statistically significant.

3. Results

Clinical characteristics of the study population were presented in detail in our previous study.

Table 1 shows ocular abnormalities and severity of ocular injuries in SM-exposed group. Majority of SM-exposed individuals had abnormalities in the lids (anterior blepharitis, 65.63%, MGD, 96.9%), punctal abnormality (66.41%), TBUT < 10 s (84.4%), tear meniscus height < 0.1 mm (89.9%), bulbar conjunctiva (hyperemia, 71.09%, abnormal vessels, 82.81%), limbus (ischemia, 76.6%, abnormal vessels, 72.66%) and cornea (calcium deposition, 62.50%, epithelium and stromal abnormalities, 86.72%). In addition, the majority of SM-exposed patients (60.9%) suffered from a severe degree of ocular involvement.

Fig. 1 illustrates the comparisons of the serum levels of MMP-9 and MMP-9/TIMPs complex between the control and SM-exposed groups.

The mean ± SD serum level of MMP-9 was significantly higher in the exposed group compared to that in the control group (410.18 ± 257.14 vs. 276.25 ± 189.43 ng/mL; P = 0.009) (Fig. 1A). Mean serum MMP-9 levels in the exposed patients who had MGD, blepharitis, abnormal tear meniscus, limbal ischemia, abnormal limbal vessels, corneal calcium deposition, abnormal epithelium, and stroma were significantly higher than those in the SM-exposed group without these abnormalities. Similarly, a significant increase in serum MMP-9 level was found in the SM-exposed group with moderate and severe ophthalmic involvement compared to the control group (P = 0.050 and 0.005) (Table 2). No significant difference was observed regarding the tear level of MMP-9 between the SM-exposed and control groups (P = 0.24) (Fig. 1B, Table 1). However, the SM-exposed group with an abnormality in fundus had significantly higher tear levels of MMP-9 as compared to the SM-exposed group without this abnormality (P = 0.022) (Fig. 2A, Table 6).

Data presented in Fig. 1C and Fig. 1D indicate that there are no significant differences between the SM-exposed and the control groups regarding the serum levels of MMP-9/TIMP-1 complex and also MMP-9/TIMP-2 complex (P = 0.094 and 0.066). However, the SM-exposed group with abnormal TBUT (P = 0.028, Fig. 2B) and corneal calcification (P = 0.045, Fig. 2C) had respectively significant reduction and elevation in serum level of MMP-9/TIMP-1 complex compared to the SM-exposed group without these problems. Also, higher MMP-9/TIMP-1 complex level was observed in the SM-exposed group with severe ocular injuries compared to the control group (P = 0.046, Table 3). Whereas, this level has been significantly reduced in SM-exposed group with nuclear sclerosis (NS) cataract (P = 0.029) as compared to the SM-exposed group without cataract (Fig. 2D and Table 3). Serum levels of MMP-9/TIMP-2 complex in the SM-exposed group with mild ocular injuries were significantly lower than those in the control group (P = 0.016, Table 4). In addition, development of subconjunctival fibrosis (P = 0.039), cornea melting (P = 0.036), abnormal cornea epithelium (P = 0.043), NS (P = 0.017), and posterior subcapsular cataract (PS) (P = 0.040) in the SM-exposed group was significantly associated with decrease in MMP-9/TIMP-2 complex levels compared to those relationships in the control group.

Interestingly, serum levels of MMP-9/TIMP-4 complex were significantly lower in the SM-exposed group compared to the control group (P < 0.001, Fig. 1E). Besides, a significant increase in MMP-9/TIMP-4 complex levels has been identified when there was an abnormality in tear meniscus (P = 0.009) and fundus (P = 0.020) in the SM-exposed group as compared to the SM exposed group without these

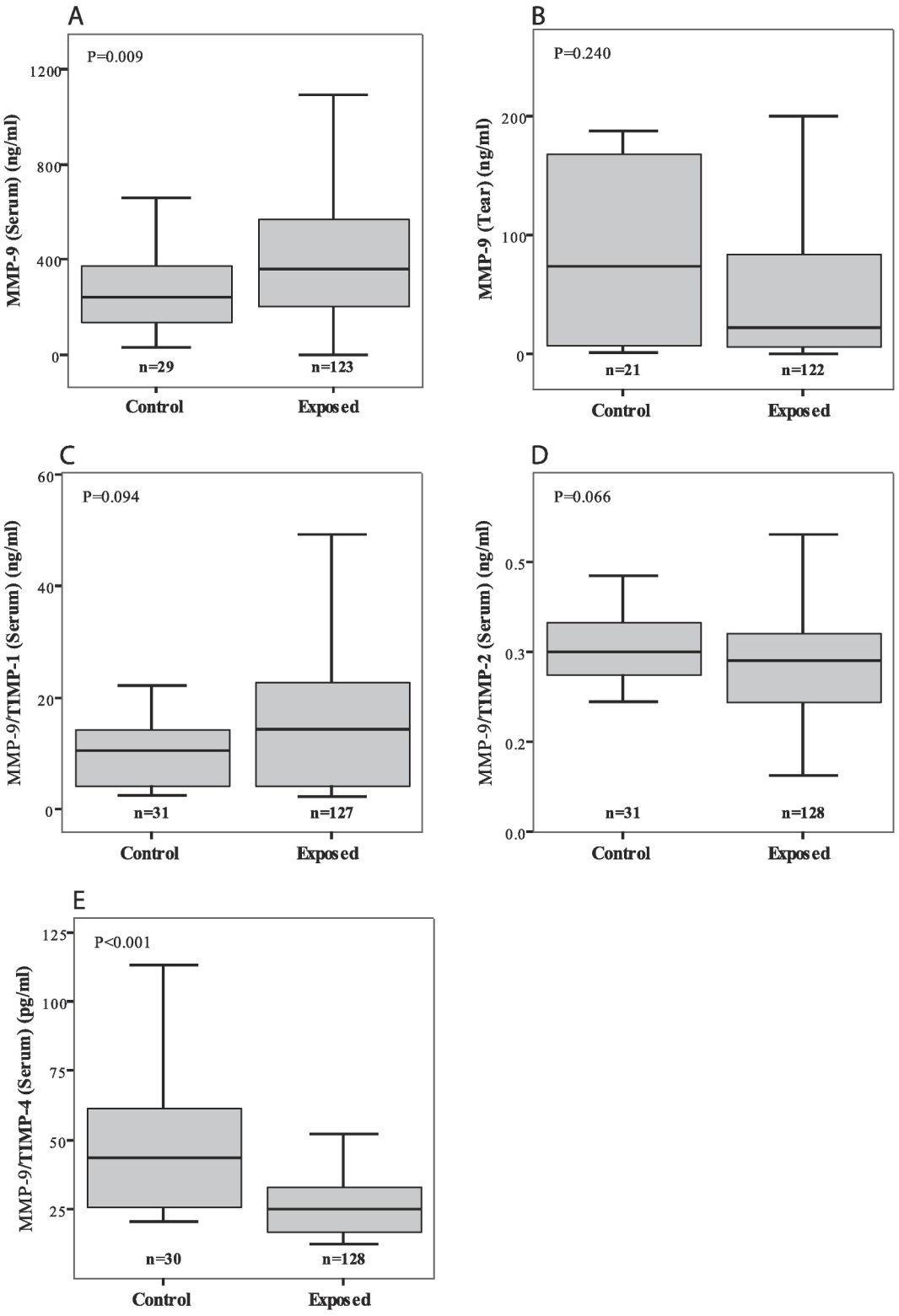

Fig. 1. Comparisons of the levels of MMP-9 and MMP-9/TIMPs complex between control and SM exposed groups. Data represented as median (first and third quartile).

problems (Fig. 2E, F and Table 5). While the difference in the index of MMP-9 to MMP-9/TIMP-1 complex was not statically significant ($P = 0.631$) in the SM-exposed and control groups, the index of MMP-9 to MMP-9/TIMP-2 complex and the index of MMP-9 to MMP-9/TIMP-4 complex were significantly high in the exposed group compared to that in the control group ($P = 0.002$ and $P < 0.001$, respectively (Fig. 3).

4. Discussion

Findings of this study showed that the majority of the exposed patients had MGD, abnormal TBUT, and bulbar conjunctival ischemia. Mean serum MMP-9 level in the SM-exposed group was significantly higher than that in the controls. However, no statistically significant

Table 2
Comparison of serum level of MMP-9 with ocular findings in Sulfur mustard group and their comparison with control group.

		MMP-9 (Serum) (μg/ml)						P-value[1]	P-value[2]	P-value[3]
		N	Mean	SD	Median	Q1	Q3			
	Control	29	0.485	0.281	0.754	0.553	0.379			
Slit Lamp - Tear status - Tear meniscus Height	> 1 mm	13	0.470	0.351	1.028	0.759	0.538	0.321		
	< 1 mm	110	0.722	0.420	1.149	0.828	0.513	**0.007**	0.505	
Slit Lamp - Tear status - TBUT	> 10″	19	0.889	0.398	1.230	0.854	0.502	**0.036**		
	< 10″	104	0.706	0.416	1.137	0.814	0.519	**0.012**	0.690	
Slit Lamp - Cornea - Ca Deposition	Normal	46	0.534	0.403	1.028	0.699	0.451	0.211		
	Abnormal	77	0.785	0.470	1.206	0.893	0.538	**0.002**	0.064	
Slit Lamp - Lens - NS Cataract	Normal	85	0.785	0.448	1.220	0.862	0.505	**0.003**		
	Abnormal	38	0.564	0.326	1.019	0.727	0.530	0.201	0.089	
Fundus	Normal	116	0.706	0.408	1.144	0.812	0.516	**0.013**		
	Abnormal	7	0.763	0.584	1.356	0.966	0.497	**0.024**	0.320	
Ophthalmic Assessment	Mild	18	0.567	0.306	1.028	0.747	0.601	0.336		
	Moderate	30	0.852	0.335	1.227	0.812	0.495	**0.050**	0.431	
	Severe	75	0.717	0.451	1.188	0.841	0.505	**0.005**	0.247	0.804

MMP-9 level was compared between all slit lamp ocular findings (normal/abnormal) subgroups with control and each other via Mann-Whitney test.
P-value[1]: Comparison of each SM-exposed subgroups with control group.
P-value[2]: Comparison of two SM-exposed subgroups based on ocular complications.
MMP: Matrix metalloproteinase.
NS: Nuclear sclerosis.
SM = Sulfur mustard.

difference was seen in the tear level of MMP-9 between the SM-exposed and control groups. Serum MMP-9 levels in SM-exposed groups were higher but, no difference was found in TIMP-1 and TIMP-2 levels. Also the serum TIMP-4 level was significantly lower in the exposed group that clearly explained the severity of systemic and ocular intoxication of the participants in the present study.

Ocular surface structures, including the lids, tear, conjunctiva, limbus, and cornea are the first line of exposure to SM during eye contact. MMPs participate in and promote the inflammation process [31]. The inflammation and microbullae formation have been reported following nitrogen mustard and SM exposure in the ocular tissue [32]. Compromised corneas by SM exposure exhibit chronic inflammation and increased MMP activity [33]. MMPs (gelatinases), mainly MMP-9 and MMP-2, implicated in vesicant injury and blistering diseases, are correlated with the remodeling at the basement membrane zone and disrupting the epidermal-dermal junction [32,33]. Rabbit cornea exposed to SM vapor shows elevated MMP-9 and MMP-2 activities during acute and late corneal involvement. Also, tear fluid levels of MMP-9 show high activity during acute to the late phase of toxicity [34]. Shohrti et al. in a group of SM-exposed patients showed that serum levels of MMP-9 were significantly higher than those in the control group [2]. Furthermore, Pourfarzam et al. found that the serum level of MMP-9 was significantly higher in SM-exposed patients with more severe pulmonary complications, while MMP-9 was significantly lower in the sputum of patients suffering from hemoptysis [35].

Beside, Ghaffarpour et al. reported that serum level of MMP-9 increased in SM-exposed group with moderate to severe pulmonary complication compared with that in the exposed group with a healthy lung, while just MMP-9/TIMP-4 complex elevated in SM-exposed group with normal lung individuals compared to that in the control group [36].

These findings are consistent with the findings of this study. It has been found that decreased MMP-9 activity in humans is associated with accelerated wound healing. So, MMP-9 could be a potential target of therapy for SM-induced ocular injury [37].

Different phases of SM-induced ocular toxicity are characterized by the following stages: immediate phase with photophobia, acute phase with corneal erosion with anterior segment inflammation, and delayed phase with epithelial defects, corneal neovascularization, and visual loss. In the delayed phase, impaired cornea accompanies with chronic inflammation, increased corneal tissue MMP activity, and limbal damage [38].

Some ocular surface inflammatory disorders showed similar clinical and immunological presentations to SM. Tear pro-MMP-9 levels increase in blepharitis, allergic eye disease, and dry eye patients [30]. In conjunctivochalasis or recurrence of pterygium, MMPs expression increases [39,40]. In ocular rosacea, tear MMP-9 and TIMP-1 activity increase and cause ocular surface irritation, erosions, and vascularization [41,42]. In surgically-induced necrotizing scleritis, increased tear fluid MMP-9 levels may suggest disease activity [43]. Tissues and cellular MMPs are effective in the development of some abnormal conditions such as corneal ulcers, bullous keratopathy, and keratoconus [44]. Acera et al. reported that in patients with blepharitis, allergic eye disease, dry eye, and conjunctivochalasis, tear pro-MMP-9 levels were significantly elevated [30]. Solomon et al. reported that MMP-9 activity, as a physiological activator of IL-1β, significantly increased in the tear fluid in dry eye patients with MGD or Sjogren's syndrome compared with that in normal subjects [45].

Sakimoto, T et al. reported that tear fluid samples of patients with peripheral noninfectious corneal ulcers showed significantly higher levels of MMP-8 and MMP-9 while TIMPs concentrations had not changed [46]. In contrast, in this study, despite significantly higher mean serum MMP-9 levels, there was no significant difference in tear MMP-9 levels between the SM-exposed and control groups. These differences with the present study may be due to altered immunological responses, possibly due to the long-lasting process of the disease and local/systemic medications for the protection of cornea against neovascularization.

In this study, mean serum levels of MMP-9/TIMP-1 and 2 complexes have shown no significant differences between the SM-exposed and control groups, but the mean serum level of MMP-9/TIMP-4 complex in the SM-exposed group was significantly higher than that in the controls. However, the SM-exposed group with abnormal TBUT had a significantly lower level of MMP-9/TIMP-1 complex while the SM-exposed group with corneal calcification and the patients with severe ocular injuries had a significantly elevated level of MMP-9/TIMP-1 complex compared with the SM-exposed group without these problems. MMP-9 is more related and inhibited by TIMP-1, but there were not any significant differences between the serum MMP-9/TIMP-1 index in the SM-exposed and control groups. Few studies have focused on the correlation between serum/tear TIMPs and ocular surface disorders. In patients with vernal keratoconjunctivitis, tear fluid MMPs, and TIMPs

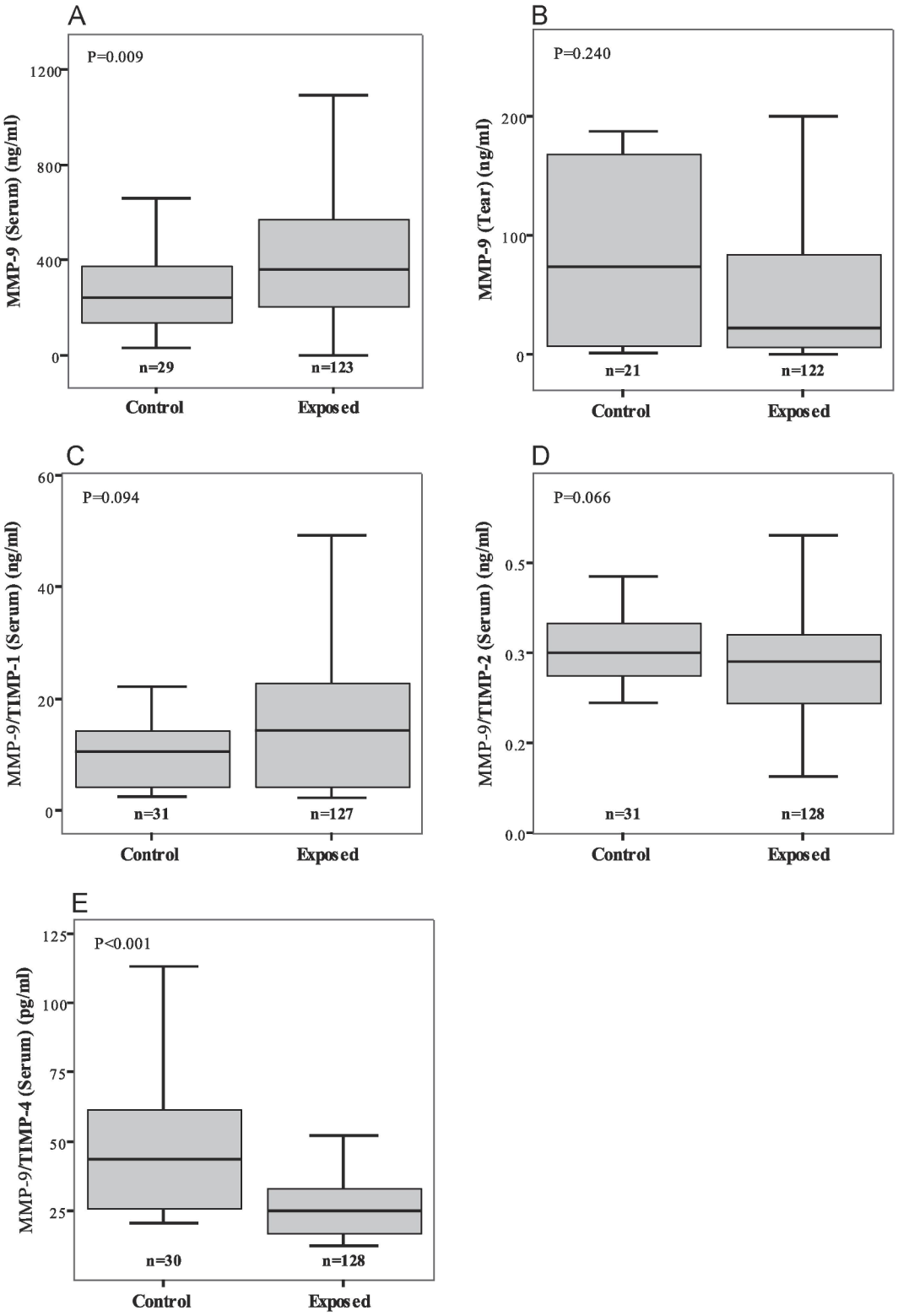

Fig. 2. Association of ocular abnormality and the levels of MMP-9 and MMP-9/TIMPs complex in SM exposed group. Data represented as and median (first and third quartile).

profiles are different from normal population [47].

Generally, MMPs and TIMPs play essential roles in the angiogenic process, including corneal neovascularization. In the normal cornea, especially in corneal epithelium, MMP-2, TIMP-1, and TIMP-2 immunoreactivities are encountered. Their activities increase after corneal injury and begin by MMP-9 and continue by MMP-2, while TIMP-1 and TIMP-2 activities gradually increase and MMPs/TIMPs ratio decreases during the healing process [48]. MMP/TIMP ratio is considered as an indicator of MMP activity, whereby higher activity is associated with a higher ratio [49]. Our finding reveals that difference in the MMP-9/TIMP-1 index was not statically significant between the SM-exposed and control groups while the MMP-9/TIMP-2 and MMP-9/TIMP-4 ratio

Table 3
Comparison of serum level of MMP9/TIMP-1 with ocular findings in the Sulfur mustard group and their comparison with the control group.

		MMP9/TIMP-1(Serum) (μg/ml)						P-value[1]	P-value[2]	P-value[3]
		N	Mean	SD	Median	Q1	Q3			
	Control	31	10.615	3.972	14.390	13.483	14.446			
Slit Lamp - Tear status - Tear meniscus Height	> 1 mm	13	14.950	11.805	30.065	22.932	20.035	0.066		
	< 1 mm	114	14.108	4.340	22.385	20.623	27.791	0.134	0.390	
Slit Lamp - Tear status - TBUT	> 10″	20	17.670	12.203	32.080	28.823	31.066	**0.007**		
	< 10″	107	14.080	4.234	21.545	19.371	26.119	0.219	**0.028**	
Slit Lamp - Cornea - Ca Deposition	Normal	47	11.580	3.812	20.070	14.711	14.785	0.706		
	Abnormal	80	15.610	4.565	24.900	24.472	31.678	**0.023**	**0.045**	
Slit Lamp - Lens - NS Cataract	Normal	88	15.840	5.580	23.290	23.320	29.660	**0.016**		
	Abnormal	39	7.430	3.840	18.440	15.308	19.117	0.864	**0.029**	
Fundus	Normal	118	14.200	4.278	22.385	19.942	26.503	0.110		
	Abnormal	9	16.310	4.479	54.850	32.895	32.726	0.190	0.372	
Ophthalmic Assessment	Mild	19	11.460	4.251	22.385	20.574	33.672	0.516		
	Moderate	30	13.618	3.812	22.915	18.309	20.830	0.445	0.902	
	Severe	78	15.743	4.651	23.350	21.910	27.653	**0.046**	0.304	0.313

MMP/TIMP-1level was compared between all slit lamp ocular findings (normal/abnormal) subgroups with control and each other via Mann-Whitney test.
P-value[1]: Comparison of each SM-exposed subgroups with control group.
P-value[2]: Comparison of two SM-exposed subgroups based on ocular complications.
MMP: Matrix metalloproteinase.
TIMP: Tissue Inhibitor of Metalloproteinase.
SM: Sulfur mustard.
Ca: Calcium.
NS: Nuclear sclerosis.

significantly increased in the SM-exposed group. The study on diabetic patients with retinopathy also displays elevated systemic values of MMP-9 and MMP-9/TIMP-1 ratio when compared to the patients without retinopathy [50]. We could not find any study to evaluate the serum ratio of MMP-9/TIMPs in patients with delayed complications of SM.

Overexpression of MMPs (MMP-2 and MMP-9) and TIMP-1 has been reported in corneal epithelial cells and retinal microvascular cells when treated with high glucose levels in vitro [51]. In another survey, analysis of gene expression of *TIMP-1*, *TIMP-2*, and *TIMP-3* in the epithelial or stromal cells revealed no significant changes in non-diabetic retinopathy and diabetic retinopathy groups [52]. The other studies revealed the serum TIMP-1, and TIMP-2 levels in chemically-injured people have shown no significant difference with normal people that are consistent with the present findings [2,53].

The results of the present work clearly shows that serum levels of MMP-9 has increased in the SM-exposed patients with moderate and severe ophthalmic complications, and TIMP-1 level was higher in patients with severe ophthalmic problems as compared to the SM-exposed people with mild and moderate ocular complications, that presumably reflect their enhanced synthesis and release from ocular tissues.

The level of TIMP-1 has reduced in the SM-exposed group with nuclear sclerotic and cortical cataract as compared to the SM-exposed group without cataract. Probably excessive remodeling in lens fiber by MMPs is one of the processes involved in cataract formation [54]. The decrease in TIMP-1 as MMP-9 inhibitor may contribute to the altered

Table 4
Comparison of serum level of MMP9/TIMP-2 with ocular findings in the Sulfur mustard group and their comparison with the control group.

		MMP9/TIMP-2 (Serum) (μg/ml)						P-value[1]	P-value[2]	P-value[3]
		N	Mean	SD	Median	Q1	Q3			
	Control	31	0.301	0.263	0.352	0.593	1.108			
Slit Lamp - Tear status - Tear meniscus Height	> 1 mm	13	0.231	0.213	0.265	0.242	0.079	**0.007**		
	< 1 mm	115	0.292	0.222	0.337	0.533	1.512	0.128	0.124	
Slit Lamp - Tear status - TBUT	> 10″	20	0.284	0.191	0.374	0.856	2.553	0.259		
	< 10″	108	0.287	0.222	0.332	0.439	1.123	0.065	0.821	
Slit Lamp - Cornea - Ca Deposition	Normal	48	0.299	0.226	0.335	0.281	0.076	0.161		
	Abnormal	80	0.281	0.196	0.331	0.638	1.806	0.062	0.645	
Slit Lamp - Lens - NS Cataract	Normal	88	0.297	0.222	0.343	0.556	1.633	0.189		
	Abnormal	40	0.264	0.210	0.322	0.390	0.859	**0.017**	0.153	
Fundus	Normal	119	0.285	0.215	0.332	0.514	1.487	0.052		
	Abnormal	9	0.314	0.245	0.349	0.373	0.236	0.871	0.217	
Ophthalmic Assessment	Mild	19	0.251	0.222	0.332	0.260	0.070	**0.016**		
	Moderate	31	0.296	0.205	0.372	0.348	0.300	0.269	0.412	
	Severe	78	0.292	0.222	0.329	0.625	1.823	0.132	0.361	0.770

MMP/TIMP-2 level was compared between all slit lamp ocular findings (normal/abnormal) subgroups with control and each other via Mann-Whitney test.
P-value[1]: Comparison of each SM-exposed subgroups with control group.
P-value[2]: Comparison of two SM-exposed subgroups based on ocular complications.
MMP: Matrix metalloproteinase.
TIMP: Tissue Inhibitor of Metalloproteinase.
TBUT: Tear break up time.
Ca: Calcium.
NS: Nuclear sclerosis.

Table 5
Comparison of serum level of MMP9/TIMP-4 with ocular findings in the Sulfur mustard group and their comparison with the control group.

		MMP9/TIMP-4(Serum) (ng/ml)						P-value[1]	P-value[2]	P-value[3]
		N	Mean	SD	Median	Q1	Q3			
	Control	30	43.405	26.100	61.820	73.275	102.326			
Slit Lamp - Tear status - Tear meniscus Height	> 1 mm	13	16.920	16.220	24.050	20.010	6.077	**0.000**		
	< 1 mm	115	26.070	17.470	33.920	88.504	358.558	**0.000**	**0.009**	
Slit Lamp - Tear status - TBUT	> 10″	20	24.450	17.100	33.610	38.615	57.377	**0.001**		
	< 10″	108	25.365	17.045	33.390	89.498	369.457	**0.000**	0.880	
Slit Lamp - Cornea - Ca Deposition	Normal	48	24.605	16.710	33.490	28.651	17.239	**0.000**		
	Abnormal	80	25.130	17.345	33.205	113.286	428.165	**0.000**	0.583	
Slit Lamp - Lens - NS Cataract	Normal	88	25.315	17.155	33.920	94.105	394.390	**0.000**		
	Abnormal	40	24.710	16.895	29.940	53.922	170.582	**0.000**	0.476	
Fundus	Normal	119	24.650	16.920	32.870	83.178	352.449	**0.000**		
	Abnormal	9	33.920	28.840	47.640	59.988	78.317	0.463	**0.020**	
Ophthalmic Assessment	Mild	19	24.650	16.630	27.980	168.794	623.011	**0.002**		
	Moderate	31	24.870	17.410	35.700	35.257	44.512	**0.001**	0.555	
	Severe	78	25.180	16.920	33.580	78.693	311.132	**0.000**	0.601	0.799

MMP/TIMP-4 level was compared between all slit lamp ocular findings (normal/abnormal) subgroups with control and each other via Mann-Whitney test.
P-value[1]: Comparison of each SM-exposed subgroups with control group.
P-value[2]: Comparison of two SM-exposed subgroups based on ocular complications.
MMP: Matrix metalloproteinase.
TIMP: Tissue Inhibitor of Metalloproteinase.
TBUT: Tear break up time.
Ca: Calcium.
NS: Nuclear sclerosis.

Table 6
Comparison of tear level of MMP-9 with ocular findings in Sulfur mustard group and their comparison with control group.

		MMP-9 (Tear) (μg/ml)						P-value[1]	P-value[2]
		N	Mean	SD	Median	Q1	Q3		
	Control	21	0.913	0.087	2.108	1.002	0.945		
Slit Lamp - Tear status - Tear meniscus Height	> 1 mm	13	0.128	0.053	0.515	0.430	0.729	0.086	
	< 1 mm	109	0.282	0.078	1.090	0.769	0.963	0.319	0.249
Slit Lamp - Tear status - TBUT	> 10″	20	0.166	0.032	0.766	0.628	0.943	0.144	
	< 10″	102	0.293	0.079	1.074	0.754	0.947	0.313	0.240
Fundus	Normal	115	0.232	0.067	0.866	0.678	0.901	0.154	
	Abnormal	7	2.113	0.215	2.630	1.633	1.238	0.106	**0.022**
Slit Lamp - Cornea - Ca Deposition	Normal	45	0.289	0.079	0.787	0.659	0.849	0.262	
	Abnormal	77	0.274	0.053	1.261	0.777	0.998	0.281	0.738
Slit Lamp - Lens - NS Cataract	Normal	85	0.282	0.070	0.747	0.727	0.974	0.265	
	Abnormal	37	0.215	0.079	1.090	0.747	0.883	0.289	0.841

MMP-9 level was compared between all slit lamp ocular findings (normal/abnormal) subgroups with control and each other via Mann-Whitney test.
P-value[1]: Comparison of each SM-exposed subgroups with control group.
P-value[2]: Comparison of two SM-exposed subgroups based on ocular complications.
MMP: Matrix metalloproteinase.
TBUT: Tear break up time.
Ca: Calcium.
NS: Nuclear sclerosis.

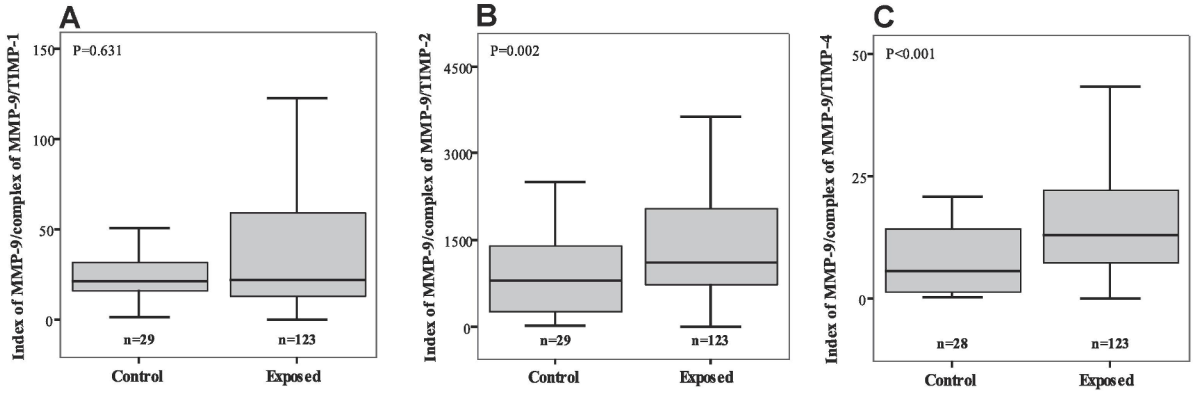

Fig. 3. Index of MMP-9 to MMP-9/TIMP-1 complex in the SM exposed and control group. Data represented as and median (first and third quartile).

lens growth and intracellular β-crystallin aggregation that characterizes human cortical cataract.

The strength point of this study is the numbers of the patients and the severity of their systemic and ocular involvement. The most important limitation was the low volume of the tear in these groups of patients. The future horizon should include the therapeutic goals focused on the basic pathophysiologic and immunologic findings in these groups of patients.

5. Conclusion

Despite increased serum MMP-9 levels in SM-exposed groups, no difference was found in TIMP-1 and TIMP-2 levels. Also, a drastic decrease in TIMP-4 was observed in the exposed group that clearly explained the severity of systemic and ocular intoxication of the participants in the present study.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105812.

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Declaration of interest

All of the authors declared no conflict of interest.

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