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Angiogenesis modulatory factors in subjects with chronic ocular complications of Sulfur Mustard exposure: A case-control study

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ABSTRACT

Background: Chronic ocular complications of Sulfur Mustard (SM) exposure leads to severe ocular morbidity during time. The aim of this study was to compare serum levels of Interleukin 17 (IL-17), IL-12, vascular endothelial growth factor (VEGF)-C, VEGF-D and nitric oxide (NO) in SM-exposed patients versus the control group and to measure tear concentration of VEGF-C only in the SM-exposed group.

Methods: In this prospective case control, 128 SM-exposed patients and 31 healthy control subjects were included. In the case group ocular manifestations were classified to three subgroups of mild (19 cases), moderate (31 cases) and severe (78 cases) forms of disease. Serum levels of IL-17, IL-12, NO, VEGF-C and VEGF-D, in all subjects and tear concentration of VEGF-C in SM-exposed group was evaluated.

Results: All subjects were male and mean ± standard deviation (SD) of age in the case and control groups were 44.9 ± 8.8 and 40.9 ± 10.1 years, respectively. Except for significantly lower serum level of IL-17 ($p < 0.001$) and NO ($p = 0.003$), other values were not significantly different. The tear concentration of VEGF-C and serum level of IL-12 were not different between subgroups in the SM-exposed group, yet were significantly lower among those with abnormally dilated and tortuous conjunctival vessels and corneal pannus, respectively ($p = 0.01$, $p = 0.015$).

Conclusions: Exposure to SM significantly reduced serum level of IL-17 and NO in the delayed phase, yet did not influence VEGF-C; VEGF-D or IL-12.

1. Introduction

Sulfur Mustard (SM), as a devastating chemical warfare agent, has been considered as an extremely toxic agent [1] with relentless chronic ocular complications [2–5].

Pathological changes of tear concentration of Vascular Endothelial Growth Factor (VEGF) level was found in SM-exposed patients as the most dominant biological route in related ocular injuries [3]. Furthermore, tear concentrations of growth factors were significantly higher among SM-exposed patients with corneal neovascularization [4]. In addition, VEGF-A, VEGF-C and VEGF-D were observed in lymphangiogenesis and angiogenesis [6,7], in the cornea. Targeting these growth factors was considered for suppressing corneal angiogenesis to enhance graft survival. A growing amount of evidence has revealed the angiogenesis [8,9] potency of Interleukin 17 (IL-17) and its pro-lymphangiogenic function in experimental cornea by increasing VEGF-A, VEGF-C and VEGF-D expression [10]. For instance, in dry eye disease, IL-17 could induce pro-inflammatory cytokines and matrix metalloproteinases release and subsequent corneal epithelial damage [11].

In addition, several evidences revealed that nitric oxide (NO) by means of its physiological effect as vasodilation could have role in physiological and pathological angiogenesis. Also, NO has prominent outcome on VEGF growth promoting effect for instance in vivo angiogenesis effect of VEGF stopped by L-NMMA as NO synthase (NOS) inhibitor [12]. Conversely, effect of NO on subretinal neovascularization has shown to be independent of VEGF pathway. As, deficiency of any isoforms of NOS significantly reduced subretinal neovascularization without alteration in VEGF expression [13]. Moreover, endogenous

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angiogenesis inhibitors act their anti-angiogenesis function using interleukin 12 (IL-12) as elimination of the IL-12 signal cascade by removal of either the IL-12 production ability or respond to IL-12 completely canceled their angiogenesis inhibitory effect [14]. IL-12 induced inhibition of angiogenesis mediated by IP-10 which inhibits endothelial cell proliferation [15]. Anti-angiogenesis effect of IL-12 in cancer and autoimmune disease therapy has been investigated in recent studies [16–19].

The aim of this study was to investigate serum level VEGF-C and VEGF-D as stimulators of angiogenesis and lymphangiogenesis [20], as well as IL-17, IL-12, and NO, the possible angiogenesis modulators [12–14,21,22] in SM-exposed patients versus the control group. In addition, tear concentration of VEGF-C was evaluated in the SM-exposed group.

2. Materials and methods

2.1. Study design and subjects

In this case-control study conducted as part of Mashhad's ocular screening program for chemical warfare male patients, 128 SM-exposed subjects aged of 21 to 75 years and 31 healthy age/gender-matched control subjects selected from patients' companions with age range of 25 to 73 years, were recruited. In the control group, subjects with normal ocular examination with no evidence of dry eye disease, no history of congenital or acquired ocular or systemic diseases and no history of ocular surgery were included. In the case group, all cases exposed to SM during Iraq-Iran war (1983–1987) and had delayed ocular complications with no history of congenital or acquired ocular or systemic diseases or malignancies other than SM-induced ocular complications, were included. Based on the severity of ocular manifestations following 19 to 25 years of SM exposure, patients were classified to three subgroups of mild (19 cases), moderate (31 cases) and severe (78 cases) forms of the disease according to chart of the foundation of martyrs and veterans affairs (Iranian ophthalmic committee of chemical warfare veterans) [23,24]. A comprehensive ocular and clinical evaluation, including assessment of the severity of ocular symptoms and signs conducted by an expert ophthalmologist, according to the earlier definition for classification of ocular involvement, was performed for both groups. Ocular surface condition divided into three subgroups: mild, moderate, and severe in SM-exposed group. Most patients were in the severe SM-induced ocular complications group, followed by moderate and mild forms.

2.2. Ethical considerations

The study protocol was approved by the Ethics Board of the Immunoregulation Research Center (IRRC) and ethical approval was received. Signed informed consent was received from all the study subjects following explanation of the steps of the study in detail and after fulfilling the inclusion and exclusion criteria. The Declaration of Helsinki was considered.

2.3. Clinical evaluation

All subjects were asked about common ocular symptoms, including blurred vision, foreign body and burning sensation, tearing, red eye, photophobia, etc., followed by a comprehensive anterior and posterior segments examination using slit-lamp (Nidek, Gamagori, Japan), direct ophthalmoscope (Heine BETA 200® ophthalmoscope, Heine, Germany) by an ophthalmologist. Thereafter, a pre-designed examination form and questionnaire was completed for each subject, according to the symptoms reported by each person as well as the findings found in the examination.

2.4. Serum collection

Peripheral blood samples were collected in a vacutainer tube (BD Biosciences). Following centrifugation for 20 min at 2000 $\times g$ (4 °C), patient's sera were extracted, aliquoted and saved at −80 °C for measurement of blood markers in the future.

2.5. Tear collection

After instillation of one drop (200 μL) of sterile 5% sodium chloride in both eyes' inferior fornices, tear samples were collected and prepared as described before [25] to be stored at −80 °C for measurement of tear VEGF-C concentration in the future.

2.6. Measuring cytokines level

IL-17, IL-12, VEGF-C, and VEGF-D DuoSet ELISA kits were obtained from R&D Systems (Minneapolis, MN, USA). Using the ELISA method, in accordance with the manufacturer's instructions, the serum levels of IL-17, IL-12, VEGF-C, and VEGF-D as well as tear level of VEGF-C were detected. Stat-Fax 2100 and Stat-Fax 2600 (USA) were used as ELISA reader and washer, correspondingly.

2.7. Nitrite assay

Based on the Griess reaction, nitrite level was measured in the serum using a Cayman product kit (USA). An equal volume of the serum sample (100 μL) and Griess reagent (a solution of 1% sulfanilamide, 5% phosphoric acid and 0.1% naphthalene diamine dihydrochloride) were mixed and incubated at room temperature for 10 min. The absorbance was measured in a plate reader at 540 nm (Stat-Fax 2100). Nitrite concentration of the unknown samples was determined using a standard curve generated by serial dilution of sodium nitrite.

2.8. Statistical analysis

Data were analyzed using the SPSS software (version 22.0, SPSS, Inc., Chicago, IL). Due to non-normally distributed data; comparison of cytokine level between the study groups was performed by nonparametric Mann-Whitney *U* test. Correlation between the IL-12, NO, and IL-17 with VEGF-C and D was computed using spearman correlation. The significance level was defined as p-values < 0.05.

3. Results

All the study subjects were male and mean ± standard deviation (SD) of age in case and control groups were 44.9 ± 8.8 and 40.9 ± 10.1 years, respectively, with an insignificant age distribution difference between the two study groups; 28 (90.3%) individuals in the control group and 104 (82.5%) in the SM-exposed group were below 50 years old (p-value = 0.289).

3.1. Local and systemic release of VEGF-C and VEGF-D

Table 1 shows the presence of ocular surface complications among the SM-exposed group. A total of 128 SM-exposed patients had an ocular complication, divided in three subgroups which were severe in 60.9%, moderate in 24.2% and mild in 14.8%, yet none of the controls had this complication.

Table 2 reveals serum VEGF-D levels in the both groups, in three subgroups of SM-exposed patients as well as in SM-exposed patients with ocular surface complications. As shown in this table, level of this cytokine was not significantly different between the control and exposed groups (p = 0.342), between the three subgroups of SM-exposed patients (mild-moderate p = 0.670; mild-severe p = 0.584 and moderate-severe p = 0.138), and between the SM-exposed patients with

Table 1
Presence of ocular surface complications in the study groups.

		n	%
Group	Control	31	19.5%
	Exposed	128	80.5%
Abnormally dilated and tortuous conjunctival vessels	No	22	17.2%
	Yes	106	82.8%
Abnormal limbal vessels	No	35	27.3%
	Yes	93	72.7%
Corneal pannus	No	87	68.0%
	Yes	41	32.0%
Corneal melting	No	70	54.7%
	Yes	58	45.3%
Severity of ophthalmic assessment	Normal	0	0.0%
	Mild	19	14.8%
	Moderate	31	24.2%
	Severe	78	60.9%

n: number; %: percentage.

and without different ocular complications, except being significantly lower in SM-exposed with corneal pannus (p = 0.025); with median and quartiles of 10.400 ng/mL (Q1: 4.457, Q3: 22.286) and 22.286 ng/mL (Q1: 5.943, Q3: 29.714) in subjects with and without corneal pannus, respectively. Although, this difference was statistically insignificant in SM-exposed patients with corneal pannus compared to the control group (p = 0.058), but clinically was noticeable.

Table 3 shows serum VEGF-C levels in the both groups, in three subgroups of SM-exposed patients as well as in SM-exposed patients with ocular surface complications. As shown in this table, level of this cytokine was not significantly different between the control and exposed groups (p = 0.356), between the three subgroups of SM-exposed patients (mild-moderate p = 0.278; mild-severe p = 0.609 and moderate-severe p = 0.331), and between SM-exposed patients with and without different ocular complications.

Table 4 shows tear VEGF-C concentration in three subgroups of SM-exposed patients as well as in SM-exposed patients with ocular surface complications. As shown in this table, level of this cytokine was not significantly different between the three subgroups of SM-exposed patients (mild-moderate p = 0.292; mild-severe p = 0.785 and moderate-severe p = 0.124), and between SM-exposed patients with and without different ocular complications, except being significantly lower in SM-exposed cases with abnormally dilated and tortuous conjunctival vessels (p = 0.011); with median and quartiles of 27.340 ng/mL (Q1: 0.00, Q3: 100.69) and 104.170 ng/mL (Q1: 58.59, Q3: 190.97) in subjects with and without abnormally dilated and tortuous conjunctival vessels, respectively.

3.2. Serum levels of IL-17 in SM-exposed patients with ocular complications

Table 5 shows serum IL-17 levels in the both groups, in three subgroups of SM-exposed patients as well as in SM-exposed patients with ocular surface complications. As shown in this table, level of this cytokine was significantly lower in exposed cases compared to the control group (p < 0.001), yet not between the three subgroups of SM-exposed patients (mild-moderate p = 0.356; mild-severe p = 0.858, and moderate-severe p = 0.448), and SM-exposed patients with and without different ocular complications.

3.3. Serum levels of IL-12 and NO in SM-exposed patients with ocular complications

Serum levels of IL-12 were investigated in SM-exposed patients and control group using ELISA. There was no significant change between both study groups (p = 0.464, Table 6). For the further investigation, the serum level of IL-12 was evaluated in SM-exposed objects with or without different ocular complications and only patients with corneal pannus showed the lower level of IL-12 compared with the patients without this complication (p = 0.015, Table 6), with median and quartiles of 0.00 ng/mL (Q1: 0.00, Q3: 116.10) and 33.290 ng/mL (Q1: 0.00, Q3: 196.00), respectively. In addition, the concentration of this cytokine was analyzed between the three subgroups of SM-exposed patients and no significant changes were found (mild-moderate, p = 0.434; mild-severe, p = 0.981, and moderate-severe p = 0.333, Table 6). The serum level of NO in both study groups presented in Table 7. Compared to the control group, SM-exposed group displayed a significantly lower level of NO (p = 0.003, Table 7). However, NO levels were not significantly different between patients with or without different ocular complications and the three subgroups of SM-exposed patients (mild-moderate, p = 0.599; mild-severe, p = 0.795, and moderate-severe p = 0.223, Table 7).

3.4. Correlation between the serum levels of IL-12, NO, and IL-17 with angiogenesis stimulators

Correlation between the serum levels of IL-12, NO, and IL-17 with

Table 2
Serum VEGF-D level in the both study groups.

		VEGF-D (serum) (pg/mL)						
		n	Median	Q1	Q3	p-Value1	p-Value2	p-Value3
Group	Control	25	20.800	7.429	29.714			
	Exposed	110	18.572	5.943	28.229	0.342		
Abnormally dilated and tortuous conjunctival vessels	No	19	20.800	5.943	28.229	0.661		
	Yes	91	17.829	4.457	28.229	0.316	0.663	
Abnormal limbal vessels	No	28	22.286	6.686	38.282	0.957		
	Yes	82	15.600	4.457	25.257	0.225	0.145	
Corneal pannus	No	73	22.286	5.943	29.714	0.772		
	Yes	37	10.400	4.457	22.286	0.058	**0.025**	
Corneal melting	No	61	16.343	4.457	28.229	0.338		
	Yes	49	19.314	5.943	28.229	0.443	0.845	
Severity of ophthalmic assessment	Mild	14	20.800	7.429	29.714	0.696		
	Moderate	26	22.286	5.943	46.850	0.910	0.670	
	Severe	70	16.343	4.457	25.257	0.210	0.584	0.138

VEGF-D: Vascular endothelial growth factor D; pg/mL: pico-grams per milliliter; n: number. p-value < 0.05 in bold (Mann–Whitney).
Q1: First quartile.
Q3: Third quartile.
p1: Comparison with the control group.
p2: Between SM-exposed with and without ocular complications.
p3: Between moderate and severe SM-exposed subgroups.

Table 3
Serum VEGF-C level in the both study groups.

		VEGF-C (serum) (pg/mL)						
		n	Median	Q1	Q3	p-Value1	p-Value2	p-Value3
Group	Control	22	1700.00	1448.78	2006.17			
	Exposed	107	1892.68	1412.06	2166.67	0.356		
Abnormally dilated and tortuous conjunctival vessels	No	20	1816.94	1515.92	2099.70	0.696		
	Yes	87	1921.95	1376.88	2166.67	0.319	0.562	
Abnormal limbal vessels	No	30	1789.30	1341.71	2165.38	0.934		
	Yes	77	1912.20	1482.93	2166.67	0.229	0.392	
Corneal pannus	No	72	1886.04	1433.01	2155.04	0.358		
	Yes	35	1904.52	1311.56	2191.21	0.466	0.958	
Corneal melting	No	60	1868.45	1329.27	2237.73	0.608		
	Yes	47	1921.95	1587.94	2160.49	0.205	0.568	
Severity of ophthalmic assessment	Mild	17	1912.20	1246.23	2144.7	0.856		
	Moderate	27	2018.52	1658.29	2299.74	0.115	0.278	
	Severe	63	1844.22	1422.11	2134.37	0.531	0.609	0.331

VEGF-C: vascular endothelial growth factor C; pg/mL: pico-grams per milliliter; n: number. p-value (Mann–Whitney).
Q1: First quartile.
Q3: Third quartile.
p1: Comparison with the control group.
p2: Between SM-exposed with and without ocular complications.
p3: Between moderate and severe SM-exposed subgroups.

the serum levels of VEGF-C and VEGF-D in both study groups and with the tear concentrations of VEGF-C in SM-exposed group were investigated (Tables 8, 9, and 10). No significant correlation between IL-12 and NO with the serum levels of VEGF-C and VEGF-D and the tear concentrations of VEGF-C was observed in SM-exposed group (Tables 8 and 10). Similar results about the correlation between IL-12 and NO with angiogenesis stimulators were found in the control group (Tables 8 and 9). However, Serum levels of IL-17 showed direct correlation with IL-12 ($r = 0.447$, $p = 0.012$) and VEGF-D ($r = 0.468$, $p = 0.018$) in the control group (Table 9). Although, there was no significant correlation of IL-17 with VEGF-C, VEGF-D, IL-12, and NO in the SM-exposed group (Table 10).

4. Discussion

In this case-control study, 128 SM-exposed patients and 31 healthy control subjects were included. In the case group, based on the severity of ocular manifestations, patients were classified into three subgroups of mild (19 cases), moderate (31 cases) and severe (78 cases) forms of the disease. Serum levels of VEGF-C, VEGF-D, IL-17, IL-12, and NO in all participants, and tear concentration of VEGF-C in the SM-exposed group were determined. All the study subjects were male and mean ± SD of age in the case and control groups were 44.9 ± 8.8 and 40.9 ± 10.1 years, respectively. Except for a decreased serum level of IL-17, and NO in SM-exposed group, other values were not significantly different between the study groups and between the three subgroups in the SM-exposed group. The tear concentration of VEGF-C was not different between the subgroups in the SM-exposed group, yet was significantly ($p = 0.01$) lower among those with abnormally dilated and tortuous conjunctival vessels.

Studies have discussed the major role of VEGF signaling in pathological angiogenesis [26]. Disruption in the balance of VEGF related pathways is highly associated with corneal angiogenesis, a typical clinical feature in various corneal disorders. Recently, anti-VEGF therapy is an extremely effective treatment for a number of patients with blinding ocular disorders [27]. Panahi et al. in their cross-sectional study [28] evaluated 25 eyes from 18 patients with chemical injury of ocular complications of SM exposure. They measured total protein and

Table 4
Tear VEGF-C level in SM-exposed patients.

		VEGF-C (tear) (pg/mL)					
		n	Median	Q1	Q3	p-Value1	p-Value2
Group	Control	0					
	Exposed	75	39.06	0.00	111.11		
Abnormally dilated and tortuous conjunctival vessels	No	10	104.17	58.59	190.97		
	Yes	65	27.34	0.00	100.69	**0.011**	
Abnormal limbal vessels	No	18	62.95	0.00	104.17		
	Yes	57	31.25	0.00	111.11	0.485	
Corneal pannus	No	46	37.11	0.00	100.69		
	Yes	29	53.57	7.81	138.89	0.243	
Corneal melting	No	44	54.69	0.00	127.14		
	Yes	31	23.44	0.00	86.54	0.300	
Severity of Ophthalmic Assessment	Mild	9	44.64	19.53	67.31		
	Moderate	17	86.54	23.44	149.31	0.292	
	Severe	49	27.34	0.00	104.17	0.785	0.124

VEGF-C: Vascular endothelial growth factor C; pg/mL: pico-grams per milliliter; n: number. p-value < 0.05 in bold (Mann–Whitney)
Q1: First quartile.
Q3: Third quartile.
p1: Between SM-exposed with and without ocular complications.
p2: Between moderate and severe SM-exposed subgroups.

Table 5
Serum IL-17 level in the both study groups.

		IL-17 (serum) (pg/mL)						
		N	Median	Q1	Q3	p-Value1	p-Value2	p-Value3
Group	Control	31	26.700	13.880	47.86			
	Exposed	127	9.401	7.304	12.22	< **0.001**		
Abnormally dilated and tortuous conjunctival vessels	No	21	9.401	7.407	11.32	< **0.001**		
	Yes	106	9.340	7.281	12.27	< **0.001**	0.805	
Abnormal limbal vessels	No	34	9.090	7.407	10.62	< **0.001**		
	Yes	93	9.535	7.281	12.40	< **0.001**	0.454	
Corneal pannus	No	86	9.326	7.407	12.27	< **0.001**		
	Yes	41	9.429	7.099	11.91	< **0.001**	0.628	
Corneal melting	No	69	9.646	7.485	12.27	< **0.001**		
	Yes	58	8.508	7.281	11.34	< **0.001**	0.300	
Severity of Ophthalmic Assessment	Mild	18	9.074	7.795	10.07	< **0.001**		
	Moderate	31	10.170	7.485	13.07	< **0.001**	0.356	
	Severe	78	9.090	7.164	12.22	< **0.001**	0.858	0.448

IL-17: interleukin 17; pg/mL: pico-grams per milliliter; n: number. p-Value < 0.05 in bold (Mann–Whitney).
Q1: First quartile.
Q3: Third quartile.
p1: Comparison with the control group.
p2: Between SM-exposed with and without ocular complications.
p3: Between moderate and severe SM-exposed subgroups.

Table 6
Serum IL-12 level in the both study groups.

		IL-12 (serum) (pg/mL)						
		N	Median	Q1	Q3	p-Value1	p-Value2	p-Value3
Group	Control	31	15.110	0.000	81.470			
	Exposed	127	22.315	0.000	150.800	0.464		
Abnormally dilated and tortuous conjunctival vessels	No	21	11.034	0.000	132.200	0.985		
	Yes	106	24.550	0.000	152.900	0.390	0.488	
Abnormal limbal vessels	No	34	10.620	0.000	78.870	0.484		
	Yes	93	22.900	0.000	196.000	0.291	0.224	
Corneal pannus	No	86	33.290	0.000	196.000	0.123		
	Yes	41	0.000	0.000	116.100	0.310	**0.015**	
Corneal melting	No	69	28.475	0.000	150.000	0.362		
	Yes	58	19.575	0.000	196.000	0.703	0.708	
Severity of ophthalmic assessment	Mild	18	20.880	0.000	78.870	0.676		
	Moderate	31	62.320	0.000	152.900	0.222	0.434	
	Severe	78	22.315	0.000	158.100	0.687	0.981	0.333

IL-12: interleukin 12; pg/mL: pico-grams per milliliter; n: number. p-Value < 0.05 in bold (Mann–Whitney).
Q1: First quartile.
Q3: Third quartile.
p1: Comparison with the control group.
p2: Between SM-exposed with and without ocular complications.
p3: Between moderate and severe SM-exposed subgroups.

VEGF concentrations in tear. Significantly lower total tear protein, as well as a higher level of VEGF and VEGF/total protein, was observed in the SM-exposed group compared to the healthy controls. Abbaszadeh et al. [4] in their case-control cross-sectional study observed significantly higher tear concentrations of VEGF-A 165 in SM-exposed subjects with and without corneal neovascularization and significantly elevated tear concentrations of platelet-derived growth factor-BB (PDGF-BB) and basic fibroblast growth factor (bFGF) only for those with corneal neovascularization compared to the healthy controls. In the current study, serum VEGF-D and VEGF-C values were not significantly different between the two study groups, except significantly lower serum levels of VEGF-D and tear concentrations of VEGF-C in SM-exposed patients with corneal pannus and abnormally dilated and tortuous conjunctival vessels, respectively. It could be assumed that reduction of VEGF-C and VEGF-D in SM-induced corneal complications probably is negative feedback to regulate angiogenesis in response to long-term complications.

There is increasing evidence of inflammatory mediators effects on the regulation of angiogenesis [29]. In this study, the correlation between IL-17 as an angiogenesis stimulator and IL-12 and NO as the inhibitors of neovascularization were examined. Our results demonstrated that serum levels of IL-17 significantly decreased in SM-exposed patients and it did not make any effect on presentation and severity of ocular surface complications and had insignificant correlation with IL-12, NO, VEGF-C, and VEGF-D in SM-exposed patients. IL-17 is a pro-inflammatory cytokine which expresses by several subsets immune cells especially type 17 T helper (Th17) cells [30]. While IL-17 mostly protects against infections, its dysbalance signaling system might play a crucial role in the pathogenesis of autoimmune diseases, cancer, and infectious diseases [31]. However, there is no study on the evaluation of IL-17 in SM-exposed patient with ocular complications to compare with the current study.

Although, Imani et al. [22], in their analytical cross-sectional study, found significantly increased IL-17 expression measured by flow cytometry in Peripheral Mononuclear Blood Cells (PBMNCs) of SM-exposed patients compared to the healthy controls. Likewise, Farahani et al.

Table 7
Serum NO level in the both study groups.

		Nitric oxide (serum) (μg/mL)					
		N	Median	Q1	Q3	p-Value1	p-Value2	p-Value3
Group	Control	31	1397.0	1196.0	1750.5			
	Exposed	127	1172.0	990.3	1555.0	**0.003**		
Abnormally dilated and tortuous conjunctival vessels	No	21	1161.0	972.6	1313.0	**0.010**		
	Yes	106	1177.0	1001.0	1564.0	**0.004**	0.765	
Abnormal limbal vessels	No	34	1084.0	967.0	1285.0	< **0.001**		
	Yes	93	1213.0	1011.0	1589.0	**0.018**	0.075	
Corneal pannus	No	86	1162.0	1001.0	1507.0	**0.002**		
	Yes	41	1210.0	990.3	1575.0	**0.032**	0.625	
Corneal melting	No	69	1118.0	1001.0	1575.0	**0.007**		
	Yes	58	1216.0	964.0	1405.0	**0.005**	0.813	
Severity of ophthalmic assessment	Mild	18	1087.0	1002.0	1567.0	**0.021**		
	Moderate	31	1161.0	946.0	1362.0	**0.002**	0.599	
	Severe	78	1213.0	997.2	1593.0	**0.015**	0.795	0.223

NO: nitric oxide; μg /mL: micrograms per milliliter; n: number. p-Value < 0.05 in bold (Mann–Whitney).
Q1: First quartile.
Q3: Third quartile.
p1: Comparison with the control group.
p2: Between SM-exposed with and without ocular complications.
p3: Between moderate and severe SM-exposed subgroups.

Table 8
Correlation between the serum levels of IL-12, NO with angiogenesis stimulators in the both study groups.

		Control		Exposed	
		IL-12 (serum) (pg/mL)	NO (serum) (μg/mL)	IL-12 (serum) (pg/mL)	NO (serum) (μg/mL)
VEGF-D (serum) (pg/mL)	r	0.199	0.068	−0.013	−0.113
	p-Value	0.341	0.758	0.895	0.243
VEGF-C (serum) (pg/mL)	r	0.084	0.212	−0.032	0.084
	p-Value	0.709	0.369	0.741	0.394
VEGF-C (tear) (pg/mL)	r	–	–	−0.068	0.011
	p-Value	–	–	0.561	0.925

NO: nitric oxide; IL-12: interleukin 12; VEGF-D: vascular endothelial growth factor D; VEGF-C: vascular endothelial growth factor C; μg /mL: micrograms per milliliter; pg/mL: pico-grams per milliliter. p-value (r: spearman correlation coefficient).

Table 9
Correlation between the serum levels of IL-12, NO, and IL-17 with angiogenesis stimulators in control group.

Control group		NO (serum) (μg/mL)	IL-12 (serum) (pg/mL)	IL-17 (serum) (pg/mL)	VEGF-D (serum) (pg/mL)	VEGF-C (serum) (pg/mL)
NO (serum) (μg/mL)	r		0.152	−0.239	0.068	0.212
	p-Value		0.440	0.220	0.758	0.369
IL-12 (serum) (pg/mL)	r	0.152		**0.447**	0.199	0.084
	p-Value	0.440		**0.012**	0.341	0.709
IL-17 (serum) (pg/mL)	r	−0.239	**0.447**		**0.468**	0.270
	p-Value	0.220	**0.012**		**0.018**	0.223
VEGF-D (serum) (pg/mL)	r	0.068	0.199	**0.468**		0.323
	p-Value	0.758	0.341	**0.018**		0.177
VEGF-C (serum) (pg/mL)	r	0.212	0.084	0.270	0.323	
	p-Value	0.369	0.709	0.223	0.177	

NO: nitric oxide; IL-12: interleukin 12; IL-17: interleukin 17; VEGF-D: vascular endothelial growth factor D; VEGF-C: vascular endothelial growth factor C; μg /mL: micrograms per milliliter; pg/mL: pico-grams per milliliter. p-Value < 0.05 in bold (r: spearman correlation coefficient).

[32] found a significantly higher expression of IL-17 gene in PBMNCs, using real time polymerase chain reaction, in SM-exposed patients compared to healthy controls. Farahani and Imani's works displayed the intracellular elevation of IL-17. It could suggest that PBMCs equipped with IL-17 migrate and have a function in injured tissues and don't have any influence on systemic levels. According to this suggestion, significantly higher amounts of $CD4^+$ $IL\text{-}17^+$Th17 were reported in transbronchial sections of SM-exposed compared to healthy controls [22]. On the contrary, in the current study, serum IL-17 levels were significantly lower than healthy controls. A possible justification could be the difference in the severity of exposure [2] among recruited study subjects, as in Frahani's study, SM-exposed subjects (n = 15) had one-time history of exposure > 30 years ago associated with moderate symptoms, whereas most SM-exposed patients (n = 128) of the current study had a history of more than one-time severe exposure, which was complicated by ocular involvement. Mishra et al. [33] in their animal model of SM-induced chronic lung injury found infiltration of $IL\text{-}17^+$ cells by immunohistochemical staining in the lung, which was observed in areas of inflammation or fibrosis. However, they did not measure serum levels of IL-17 in this well-designed animal model. Serum IL-17 was significantly reduced in SM-exposed subjects of the current study, but there was no histological evidence for lung IL-17 level in the subjects. Besides, during inflammatory infiltration in the inflammatory response, despite the accumulation of certain inflammatory mediators in some organs such as the lung, the serum level of inflammatory cytokines could be variable and not necessarily similarly high in accordance with its tissue level. Therefore, comparison of the mentioned animal study and the current work is not sensible. Furthermore, another justification for this discrepancy could be the difference in the amount of time elapsed since SM exposure in the current and above mentioned study, as in the animal model, the definition of chronicity was above 30 days post exposure, which is not comparable with the current case group, who had been evaluated after 19 to 25 years of SM exposure. Interestingly, our finding showed a significant correlation of IL-17 and VEGF-D in the control (r = 0.468, p = 0.018) and near to significant correlation of them in SM-exposed group (r = 0.161, p = 0.092). Although, the difference in serum VEGF-D levels was statistically insignificant in SM-exposed patients with corneal pannus compared to the control group (p = 0.058), but clinically was noticeable. IL-17 can potentiate the angiogenesis effect of VEGF [27]. Chauhan et al. [10] demonstrated the pro-lymphangiogenic function of IL-17 in the cornea of the experimental ocular disease by significant increasing VEGF-A,

Table 10
Correlation between the serum levels of IL-12, NO, and IL-17 with angiogenesis stimulators in SM-exposed group.

SM-exposed group		NO (serum) (μg/mL)	IL-12 (serum) (pg/mL)	IL-17 (serum) (pg/mL)	VEGF-D (serum) (pg/mL)	VEGF-C (serum) (pg/mL)	VEGF_C (tear) (pg/mL)
NO (serum) (μg/mL)	r		0.152	0.077	−0.113	0.084	0.011
	p-Value		0.094	0.394	0.243	0.394	0.925
IL-12 (serum) (pg/mL)	r	0.152		0.167	−0.013	−0.032	−0.068
	p-Value	0.094		0.061	0.895	0.741	0.561
IL-17 (serum) (pg/mL)	r	0.077	0.167		0.161	−0.101	−0.059
	p-Value	0.394	0.061		0.092	0.300	0.613
VEGF-D (serum) (pg/mL)	r	−0.113	−0.013	0.161		−0.075	0.048
	p-Value	0.243	0.895	0.092		0.460	0.705
VEGF-C (serum) (pg/mL)	r	0.084	−0.032	−0.101	−0.075		0.001
	p-Value	0.394	0.741	0.300	0.46		0.999
VEGF-C (tear) (pg/mL)	r	0.011	−0.068	−0.059	0.048	0.001	
	p-Value	0.925	0.561	0.613	0.705	0.999	

NO: nitric oxide; IL-12: interleukin 12; IL-17: interleukin 17; VEGF-D: vascular endothelial growth factor D; VEGF-C: vascular endothelial growth factor C; μg/mL: micrograms per milliliter; pg/mL: pico-grams per milliliter. p-value (r: spearman correlation coefficient).

VEGF-C, and VEGF-D expression. In a recently published review article, the authors underline that the role of IL-17 in neovascularization is controversial. In this paper, the functional roles of IL-17 in ocular neovascular diseases have been reviewed with emphasis on diseases involving the posterior segment such as proliferative diabetic retinopathy, age-related macular degeneration, retinopathy of prematurity, and retinal vein occlusion. The authors suggest that because of the important role that IL-17 exerts in various ways in ocular neovascular diseases, inhibition of this cytokine may be a potential treatment for inhibition of neovascularization in the retina and choroid. However, effects of IL-17 on neovascularization due to its function through different cell types, cytokines, and factors are complex, suggesting that further studies should be conducted to investigate the synergistic effects of anti-IL-17 use and anti-VEGF drugs [34]. In another review article, the role of IL-17 in the anterior segment diseases is investigated. The authors conclude that this cytokine plays an important role in the pathogenesis of ocular surface and corneal diseases, especially infective keratitis and dry eye. IL-17 neutralizing antibodies have raised hopes of reducing the severity of some diseases. Therefore, targeting it can provide useful therapies in the future, especially for ocular surface and corneal diseases [35] which are the dominant findings in the examination of the anterior segment of SM-exposed subjects. Unlike our study which evaluated serum levels of IL-17 and revealed significantly lower serum level, the vast majority of the studies cited in this review article assessed tear level of IL-17 that had increased. Therefore, further studies with measuring tear levels of this cytokine in SM-exposed subjects with different severity of ocular manifestations seem necessary. Nevertheless, presented evidence suggests that serum level of IL-17 alone had no effect on VEGF-C and VEGF-D expression in SM-exposed patients.

NO, as well as IL-12 has a remarkable role in modulating ocular neovascularization [13–19]. Also, according to previous investigations peroxynitrite formation can caused by excess amount of NO and this is associated with degenerative diseases and diabetic retinopathy [36–38]. Despite this, our result demonstrated a significant reduction of NO in SM-exposed group without any association with having ocular surface disorders and severity of ophthalmic complications. Several studies have demonstrated the anti-angiogenesis activity of IL-12 beside its immunological function [16,39]. Our study revealed that serum levels of IL-12 did not change in SM-exposed patients compared to controls. Although, in SM-exposed group only patients with corneal pannus showed the lower level of IL-12 compared with the patients without this complication. Also, insignificant correlation found between serum VEGF-D and VEGF-C as well as tear VEGF-C level with serum IL-12 level.

One of the main limitations of this study was the small sample size of each group. Therefore, lack of meaningful findings does not mean its absence. Also, our findings revealed from a case-control study and further investigations with the cohort study design could be led to more conclusive results. Angiogenesis is usually observed after SM exposure, as such, the VEGF family is predictably increased in these patients, as well established in previous publications. However, it is noticed that only IL-17 presented significant difference in SM group, however, VEGF family showed no significance, which is inconsistent with other investigations, making it even less valuable to test the serum cytokines as mentioned in this study. Therefore, further studies with measurement of tear levels of those cytokines seem to be necessary. In spite of these limitations, the strengths of this study, which included a homogenous population with a history of SM exposure and chronic ocular complications, make the findings particularly unique. Mustard gas is considered an important chemical warfare agent produced and stored in many countries and is probably the most widely distributed chemical warfare agent in the world [40]; therefore, knowing the complications of this potent chemical agent is necessary.

In conclusion, this study found a positive association between serum level of IL-17 and NO with SM exposure, yet not with VEGF-C, VEGF-D, or IL-12. Also, insignificant correlation between IL-12, IL 17, and NO with all VEGF subtypes in SM-exposed group. Possibly, serum levels of those factors are not related to immunopathogenesis of mustard gas-induced ocular manifestations; however, further longitudinal studies are required to prove this rationalization.

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Declaration of competing interest

The authors report no conflict of interest in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105843.

References

[1] E.S. Mirsharif, F. Heidary, M.R. Vaez Mahdavi, R. Gharebaghi, S. Pourfarzam, T. Ghazanfari, Sulfur mustard-induced changes in blood urea nitrogen, uric acid and creatinine levels of civilian victims, and their correlation with spirometric

values, Iran. J. Public Health 47 (11) (2018) 1725–1733 Nov. (PubMed PMID: 30581790; PubMed Central PMCID: PMC6294863).
[2] H. Ghasemi, P. Owlia, M.R. Jalali-Nadoushan, S. Pourfarzam, G. Azimi, M.E. Yarmohammadi, J. Shams, F. Fallahi, S. Moaiedmohseni, A. Moin, R. Yaraee, M.R. Vaez-Mahdavi, S. Faghihzadeh, Z. Mohammad Hassan, M.R. Soroush, M.M. Naghizadeh, S.K. Ardestani, T. Ghazanfari, A clinicopathological approach to sulfur mustard-induced organ complications: a major review, Cutan. Ocul. Toxicol. 32 (4) (2013 Oct) 304–324, https://doi.org/10.3109/15569527.2013.781615 (Epub 2013 Apr 16. Review. PubMed PMID: 23590683).
[3] Y. Panahi, A. Shahbazi, M. Naderi, K. Jadidi, A. Sahebkar, Sulfur mustard-related ocular complications: a review of proteomic alterations and pathways involved, Curr. Pharm. Des. 24 (24) (2018) 2849–2854, https://doi.org/10.2174/1381612824666180903112218 PubMed PMID: 30179120.
[4] M. Abbaszadeh, N.S. Aidenloo, M.K. Nematollahi, Q. Motarjemizadeh, Investigating the association between angiogenic cytokines and corneal neovascularization in sulfur mustard intoxicated subjects 26 years after exposure, Toxicol. Int. 21 (3) (2014) 300–306, https://doi.org/10.4103/0971-6580.155375 Sep-Dec. (PubMed PMID: 25948970; PubMed Central PMCID: PMC4413414).
[5] H. Ghasemi, A. Mostafaie, R. Yaraee, Z.M. Hassan, A. Rezaei, M. Mahmoudi, S. Faghihzadeh, M.R. Soroush, S.K. Ardestani, M. Babaei, M. Jalali-Nadoushan, A. Khamesipour, M. Ghassemi-Broumand, T. Ghazanfari, Association of serum immunoglobulins levels and eye injuries in sulfur mustard exposed: Sardasht-Iran Cohort Study, Int. Immunopharmacol. 17 (3) (2013 Nov) 944–951, https://doi.org/10.1016/j.intimp.2012.12.027 (Epub 2013 Jan 29. PubMed PMID: 23370295).
[6] Skobe M, Dana R. Blocking the path of lymphatic vessels. Nat. Med.. 2009 Sep;15(9):993–4. doi: https://doi.org/10.1038/nm0909-993. PubMed PMID: 19734869; PubMed Central PMCID: (PMC2924617).
[7] S. Goyal, S.K. Chauhan, J. El Annan, N. Nallasamy, Q. Zhang, R. Dana, Evidence of corneal lymphangiogenesis in dry eye disease: a potential link to adaptive immunity? Arch. Ophthalmol. 128 (7) (2010 Jul) 819–824, https://doi.org/10.1001/archophthalmol.2010.124 (PubMed PMID: 20625040; PubMed Central PMCID: PMC3089983).
[8] M. Numasaki, J. Fukushi, M. Ono, S.K. Narula, P.J. Zavodny, T. Kudo, P.D. Robbins, H. Tahara, M.T. Lotze, Interleukin-17 promotes angiogenesis and tumor growth, Blood 101 (7) (2003) 2620–2627. Apr 1. (Epub 2002 Oct 31. PubMed PMID: 12411307).
[9] S.R. Pickens, M.V. Volin, A.M. Mandelin 2nd, J.K. Kolls, R.M. Pope, S. Shahrara, IL-17 contributes to angiogenesis in rheumatoid arthritis, J. Immunol. 184 (6) (2010 Mar 15) 3233–3241, https://doi.org/10.4049/jimmunol.0903271 (Epub 2010 Feb 19. PubMed PMID: 20173024; PubMed Central PMCID: PMC2857761).
[10] S.K. Chauhan, Y. Jin, S. Goyal, H.S. Lee, T.A. Fuchsluger, H.K. Lee, R. Dana, A novel pro-lymphangiogenic function for Th17/IL-17, Blood 118 (17) (2011) 4630–4634, https://doi.org/10.1182/blood-2011-01-332049 Oct 27. (Epub 2011 Sep 8. PubMed PMID: 21908425; PubMed Central PMCID: PMC3208279).
[11] W. Stevenson, S.K. Chauhan, R. Dana, Dry eye disease: an immune-mediated ocular surface disorder, Arch. Ophthalmol. 130 (1) (2012) 90–100, https://doi.org/10.1001/archophthalmol.2011.364 Jan. (Review. PubMed PMID: 22232476; PubMed Central PMCID: PMC3677724).
[12] M. Ziche, L. Morbidelli, Nitric oxide and angiogenesis, J. Neuro-Oncol. 50 (1–2) (2000 Oct-Nov) 139–148 (Review. PubMed PMID: 11245273).
[13] Ando A, Yang A, Mori K, Yamada H, Yamada E, Takahashi K, Saikia J, Kim M, Melia M, Fishman M, Huang P, Campochiaro PA. Nitric oxide is proangiogenic in the retina and choroid. J. Cell. Physiol.. 2002 Apr;191(1):116–24. PubMed PMID: (11920687).
[14] A. Albini, C. Brigati, A. Ventura, G. Lorusso, M. Pinter, M. Morini, A. Mancino, A. Sica, D.M. Noonan, Angiostatin anti-angiogenesis requires IL-12: the innate immune system as a key target, J. Transl. Med. 7 (2009 Jan 14) 5, https://doi.org/10.1186/1479-5876-7-5 (PubMed PMID: 19144161; PubMed Central PMCID: PMC2630934).
[15] M. Toi, M. Gion, H. Saji, M. Asano, R. Dittadi, S. Gilberti, N. Locopo, G. Gasparini, Endogenous interleukin-12: relationship with angiogenic factors, hormone receptors and nodal status in human breast carcinoma, Int. J. Oncol. 15 (6) (1999) 1169–1175 Dec. (PubMed PMID: 10568824).
[16] M. Ali, V. Mali, S. Haddox, S.M. AbdelGhany, S.E.M. El-Deek, A. Abulfadl, K. Matrougui, S. Belmadani, Essential role of IL-12 in angiogenesis in type 2 diabetes, Am. J. Pathol. 187 (11) (2017 Nov) 2590–2601, https://doi.org/10.1016/j.ajpath.2017.07.021 (Epub 2017 Aug 22. PubMed PMID: 28837799; PubMed Central PMCID: PMC5809595).
[17] Cicchelero L, Denies S, Vanderperren K, Stock E, Van Brantegem L, de Rooster H, Sanders NN. Immunological, anti-angiogenic and clinical effects of intratumoral interleukin 12 electrogene therapy combined with metronomic cyclophosphamide in dogs with spontaneous cancer: a pilot study. Cancer Lett. 2017 Aug 1;400:205–218. doi: https://doi.org/10.1016/j.canlet.2016.09.015. (Epub 2016 Sep 28. PubMed PMID: 27693635).
[18] A. Ciomber, A. Smagur, I. Mitrus, T. Cichoń, R. Smolarczyk, A. Sochanik, S. Szala, M. Jarosz, Antitumor effects of recombinant antivascular protein ABRaA-VEGF121 combined with IL-12 gene therapy, Arch. Immunol. Ther. Exp. 62 (2) (2014 Apr) 161–168, https://doi.org/10.1007/s00005-013-0259-5 (Epub 2013 Nov 13. PubMed PMID: 24220932; PubMed Central PMCID: PMC3950566).
[19] R.M. Pope, S. Shahrara, Possible roles of IL-12-family cytokines in rheumatoid arthritis, Nat. Rev. Rheumatol. 9 (4) (2013) 252–256, https://doi.org/10.1038/nrrheum.2012.170 Apr. (Epub 2012 Oct 23. Review. PubMed PMID: 23090510).
[20] J.F. Yang, A. Walia, Y.H. Huang, K.Y. Han, M.I. Rosenblatt, D.T. Azar, J.H. Chang, Understanding lymphangiogenesis in knockout models, the cornea, and ocular diseases for the development of therapeutic interventions, Surv. Ophthalmol. 61 (3) (2016 May-Jun) 272–296, https://doi.org/10.1016/j.survophthal.2015.12.004 (Epub 2015 Dec 17. Review. PubMed PMID: 26706194; PubMed Central PMCID: PMC4835265).
[21] M. Iman, R. Rezaei, S. Azimzadeh Jamalkandi, P. Shariati, F. Kheradmand, J. Salimian, Th17/Treg immunoregulation and implications in treatment of sulfur mustard gas-induced lung diseases, Expert. Rev. Clin. Immunol. 13 (12) (2017) 1173–1188, https://doi.org/10.1080/1744666X.2017.1389646 Dec. (Epub 2017 Oct 23. Review. PubMed PMID: 28994328).
[22] S. Imani, J. Salimian, M. Bozorgmehr, E. Vahedi, A. Ghazvini, M. Ghanei, Y. Panahi, Assessment of Treg/Th17 axis role in immunopathogenesis of chronic injuries of mustard lung disease, J. Recept. Signal Transduct. Res. 36 (5) (2016 Oct) 531–541, https://doi.org/10.3109/10799893.2016.1141953 (Epub 2016 Feb 19. PubMed PMID: 26895417).
[23] Ghassemi-Broumand M, Aslani J, Emadi SN. Delayed ocular, pulmonary, and cutaneous complications of mustards in patients in the city of Sardasht, Iran. Cutan. Ocul. Toxicol.. 2008;27(4):295–305. doi: https://doi.org/10.1080/15569520802327807. PubMed PMID: (18756385).
[24] Ghasemi H, Ghazanfari T, Babaei M, Soroush MR, Yaraee R, Ghassemi-Broumand M, Javadi MA, Foroutan A, Mahdavi MR, Shams J, Pourfarzam S, Moaiedmohseni S, Nadoushan MR, Owlia P, Panahi Y, Hassan ZM, Faghihzadeh S. Long-term ocular complications of sulfur mustard in the civilian victims of Sardasht, Iran. Cutan. Ocul. Toxicol.. 2008;27(4):317–26. doi: https://doi.org/10.1080/15569520802404382. PubMed PMID:(19037764).
[25] H. Ghasemi, T. Ghazanfari, R. Yaraee, S. Pourfarzam, M.R. Soroush, S. Faghihzadeh, M. Babaei, M.M. Naghizadeh, Z. Mohammad Hassan, Evaluation of the tear and serum levels of IL-8 in sulfur mustard intoxicated patients 20 years after exposure, Cutan. Ocul. Toxicol. 31 (2) (2012 Jun) 132–137, https://doi.org/10.3109/15569527.2011.618940 (Epub 2011 Oct 4. PubMed PMID: 21967620).
[26] Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - in control of vascular function. Nat. Rev. Mol. Cell Biol.. 2006 May;7(5):359–71. Review. PubMed PMID: (16633338).
[27] A. Sene, D. Chin-Yee, R.S. Apte, Seeing through VEGF: innate and adaptive immunity in pathological angiogenesis in the eye, Trends Mol. Med. 21 (1) (2015) 43–51, https://doi.org/10.1016/j.molmed.2014.10.005 Jan. (Epub 2014 Nov 1. Review. PubMed PMID: 25457617; PubMed Central PMCID: PMC4282831).
[28] Y. Panahi, A. Eftekhari Milani, A. Sahebkar, M. Naderi, M. Babaei, F. Beiraghdar, S. Parvin, Y. Dadjo, Tear total protein analysis in patients with late sulfur mustard-induced ocular complications: a cross-sectional study, Cutan. Ocul. Toxicol. 31 (2) (2012 Jun) 104–110, https://doi.org/10.3109/15569527.2011.615359 (Epub 2011 Sep 29. PubMed PMID: 21958134).
[29] C. Costa, J. Incio, R. Soares, Angiogenesis and chronic inflammation: cause or consequence? Angiogenesis 10 (3) (2007) 149–166 (Epub 2007 Apr 25. Review. PubMed PMID: 17457680).
[30] N. Amatya, A.V. Garg, S.L. Gaffen, IL-17 signaling: the yin and the yang, Trends Immunol. 38 (5) (2017) 310–322, https://doi.org/10.1016/j.it.2017.01.006 May. (Epub 2017 Feb 20. Review. PubMed PMID: 28254169; PubMed Central PMCID: PMC5411326).
[31] K. Eyerich, V. Dimartino, A. Cavani, IL-17 and IL-22 in immunity: driving protection and pathology, Eur. J. Immunol. 47 (4) (2017 Apr) 607–614, https://doi.org/10.1002/eji.201646723 (Review. PubMed PMID: 28295238).
[32] P. Farahani, R. Halabian, E. Vahedi, J. Salimian, Increased genes expression levels of cytokines related to Th17/Treg cells in peripheral blood mononuclear cell correlate with clinical severity in COPD and mustard gas-exposed patients, Iran. J. Allergy Asthma Immunol. 16 (5) (2017) 396–403 Oct. (PubMed PMID: 29149779).
[33] N.C. Mishra, J. Rir-sima-ah, G.R. Grotendorst, R.J. Langley, S.P. Singh, S. Gundavarapu, W.M. Weber, J.C. Pena-Philippides, M.R. Duncan, M.L. Sopori, Inhalation of sulfur mustard causes long-term T cell-dependent inflammation: possible role of Th17 cells in chronic lung pathology, Int. Immunopharmacol. 13 (1) (2012) 101–108, https://doi.org/10.1016/j.intimp.2012.03.010 May. (Epub 2012 Mar 28. PubMed PMID: 22465472; PubMed Central PMCID: PMC3340497).
[34] Y. Li, Y. Zhou, Interleukin-17: the role for pathological angiogenesis in ocular neovascular diseases, Tohoku J. Exp. Med. 247 (2) (2019 Feb) 87–98, https://doi.org/10.1620/tjem.247.87 (Review. PubMed PMID: 30773517).
[35] K.B. Garbutcheon-Singh, N. Carnt, U. Pattamatta, C. Samarawickrama, A. White, V. Calder, A review of the cytokine IL-17 in ocular surface and corneal disease, Curr. Eye Res. 44 (1) (2019 Jan) 1–10, https://doi.org/10.1080/02713683.2018.1519834 (Epub 2018 Sep 19. PubMed PMID: 30230384).
[36] R. Othman, E. Vaucher, R. Couture, Bradykinin type 1 receptor - inducible nitric oxide synthase: a new axis implicated in diabetic retinopathy, Front. Pharmacol. 10 (2019) 300, https://doi.org/10.3389/fphar.2019.00300 Mar 29. (eCollection 2019. PubMed PMID: 30983997; PubMed Central PMCID: PMC6449803).
[37] Ellis EA, Guberski DL, Hutson B, Grant MB. Time course of NADH oxidase, inducible nitric oxide synthase and peroxynitrite in diabetic retinopathy in the BBZ/WOR rat. Nitric Oxide. 2002 May;6(3):295–304. PubMed PMID: (12009847).
[38] L.N. Hao, M. Wang, J.L. Ma, T. Yang, Puerarin decreases apoptosis of retinal pigment epithelial cells in diabetic rats by reducing peroxynitrite level and iNOS expression, Sheng Li Xue Bao 64 (2) (2012 Apr 25) 199–206 (PubMed PMID: 22513471).
[39] Zhou Y, Yoshida S, Kubo Y, Kobayashi Y, Nakama T, Yamaguchi M, Ishikawa K, Nakao S, Ikeda Y, Ishibashi T, Sonoda KH. Interleukin-12 inhibits pathological neovascularization in mouse model of oxygen-induced retinopathy. Sci. Rep.. 2016 Jun 17;6:28140. doi: https://doi.org/10.1038/srep28140. PubMed PMID: 27312090; PubMed Central PMCID: (PMC4911585).
[40] Szinicz L. History of chemical and biological warfare agents. Toxicology. 2005 Oct 30;214(3):167–81. PubMed PMID: (16111798).