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Peripheral blood mononuclear cellular viability and its correlation with long-term pulmonary complications after sulfur mustard exposure

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ABSTRACT

Introduction: Sulfur mustard (SM) as a chemical warfare agent has short- and long-term complications on its victims. Complications of exposure to SM depend on the level of contamination. Long-term pulmonary complications are the most serious problems. Recent evidence has shown that absorbed SM can be conducted to other tissues by the bloodstream. In this study, we evaluated the long-term effects of SM on the vital activity of peripheral blood mononuclear cells (PBMCs) in SM-exposed patients with long-term pulmonary complications.
Materials and methods: Our study samples were 110 patients with long-term pulmonary complications in the SM-exposed group and 109 unexposed individuals in the control group. After clinical examination and pulmonary function tests, the severity of pulmonary complications was classified. Also, the participants' peripheral blood was taken into EDTA-treated Vacutainer tubes. Then, the complete blood count (CBC) was calculated, and PBMCs was purified from whole blood using Ficol-Paque gradient method, finally, the vital activity was assessed by MTT assay.
Result: The vital activity of PBMCs in the SM-exposed group with the mitogen was significantly lower than that in the control group ($P = 0.016$). Whereas, there was no significant difference in the viability of PBMCs without the mitogen between two groups.

Furthermore, hematologic findings indicated that the SM-exposed group had a significant increase in the total count of WBC, neutrophil, MCV, and HCT values but the lymphocyte count and MCHC value were significantly lower than those in the control group.
Conclusion: Exposure to SM even after a long time, can affect hematologic parameters and vital activity of PBMCs.

1. Introduction

Sulfur mustard (SM), commonly known as a vesicant and an alkylating chemical agent, has been widely used in the First World War, Iraq-Iran war, and recently in Syria conflict [1,2]. After absorption, SM alkylates nucleic acids and proteins, by forming ethylene episulfonium ion intermediate and destroys cell homeostasis and ultimately leads to cell death [3]. Eye, skin, and lung are the main targets for SM toxic effects. SM has short and long-term complications [4]. While the acute effects of SM intoxication are relatively clear, but its long-term or delayed complications are still not well characterized [5]. Delayed effects of SM exposure in the skin, eye, and lung, as three main target organs, have not been entirely reported in Iranian veterans [3,6–8]. Complications of exposure to SM include various levels of cutaneous, ocular,

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gastrointestinal, hematological, immunological, reproductive, and neurological injuries, depending on the degree of contamination [9]. More than 100,000 Iranian people were exposed to SM, and some of them are still suffering from its complications [10]. As it has been reported, long-term pulmonary complications are the most basic problems of SM-exposed subjects [11,12]. The most distressing delayed pulmonary complications of SM intoxication are chronic obstructive pulmonary disease (COPD), chronic bronchitis, bronchiectasis, bronchiolitis obliterans, pulmonary hypertension, and pulmonary fibrosis [13,14]. These pulmonary complications can adversely affect the patients' quality of life. There is some evidence that absorbed SM can be conducted to other tissues by the bloodstream [15]. Therefore, it can be an attack on peripheral blood cells. There are a few studies on the effects of SM on hematological parameters that have a significant role in morbidity and mortality rate. It has been reported that 27–30 years after SM exposure, total white blood cell (WBC) count, hematocrit (HCT), and mean corpuscular volume (MCV) were significantly increased but mean corpuscular hemoglobin concentration (MCHC) was significantly decreased in SM-exposed patients compared to those items in the control group [16]. Also, another study was reported that 16–20 years after exposure, WBC, red blood cell (RBC), HCT, and the percentages of monocytes and CD3+ lymphocytes were significantly higher and the proportion of CD16 + CD56+ cells were significantly lower in the patients than the control group [3]. However, Keramati et al. showed that among hematological parameters, only the percentages of reticulocytes in SM-exposed patients, 23 years after SM exposure was significantly higher than that in the control group [15]. Furthermore, 20 years after SM-exposure, it is reported that the absolute count of natural killer (NK) cells and CD3+/CD16+56+ percentages were significantly higher, but CD45+/CD3+, CD45+/CD3+/CD4+ percentages were significantly lower than those values in the control group [17].

Several studies have been performed on the long-term complications of SM-exposure in Iranian veterans. In the present study, the long-term effect of SM on the vital activity of peripheral blood mononuclear cells (PBMCs) is evaluated. As well, hematological system should be evaluated as target organs for SM intoxication, thus we examined hematological parameters and their correlation with SM-induced delayed pulmonary complications 25 years after SM exposure.

2. Materials and method

2.1. Study design and participants

In a case-control study, 110 SM-exposed individuals based on the medical documents verified by the Medical Committee of the Foundation of Martyr and Veterans Affair, and 109 healthy individuals without any background of SM exposure, were recruited. The control group included the unexposed males matched by age with the SM-exposed group (35–60 years). Based on the documents, all the patients had long-term pulmonary complications. Chronic cough, sputum, hemoptysis, and dyspnea were considered as common pulmonary symptoms of SM exposure. No patient had contact with pollution in their workplace.

2.2. Ethical considerations

This study was performed following the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects, amended in 2013. The study was approved by the Ethics Committee of the Board of Research Ethics of Shahed University (encode Shahed.REC.1392.49). Written informed consent was obtained from all the subjects in this study.

2.3. Clinical evaluation

The complete lung examination was done by expert lung specialists. The patients' history including chronic pulmonary symptoms such as chronic cough, hemoptysis, sputum, and dyspnea severity, were taken. Chronic cough has been defined as a persistent cough for > 3 weeks. Pulmonary function of most participants was evaluated by spirometry test (Chest 801 Spirometry), allotting to the American Thoracic Society Criteria. According to the results of spirometry and clinical findings, the severity of pulmonary complications was classified based on the diagnostic protocol adopted by the Iranian Medical Committee of the Foundation of Martyrs and Veterans Affairs [18].

2.4. Complete blood count (CBC)

From every participant, 6 mL of peripheral blood was taken into EDTA-treated vacutainer tube (BD Biosciences). The complete blood count (CBC) was calculated with a cell counter (Kx21 manufactured by SYSMEX) in both control and exposed groups at the same time. Also, a portion of cells were examined by Wright-Giemsa staining.

2.5. Isolation of peripheral blood mononuclear cells using Ficol-Paque gradient

Isolation of peripheral blood mononuclear cells (PBMCs) was performed following the method described by Fuss et al. [19] with some modifications. PBMCs were purified using Ficol-Paque gradient method. Briefly, 4 mL of Ficoll-Paque gradient was pipetted into two 15-mL centrifuge tubes. The K2EDTA-blood samples were diluted 1:1 in phosphate-buffered saline (PBS) and carefully layered over the Ficoll-Paque gradient (9 to 10 mL/tube). The tubes were centrifuged for 30 min at 400*g*. The cell interface layer was harvested carefully, and the cells were washed twice in PBS (for 10 min at 300*g*) and suspended in RPMI 1640 medium with GlutaMAX supplemented with 10% FBS and penicillin (100 U/mL), and streptomycin (100 μg/mL).

2.6. Evaluation of the vital activity of PBMCs

In this study, to evaluate the cytotoxic effect of SM on PBMCs, MTT (3-(4,5-dimethylimidazole-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay was used and the percentage of surviving cells were determined [20]. Briefly, a mitochondrial succinate dehydrogenase enzyme converts the yellow salt of MTT to the purple formazan crystalline, and the product color is evaluated at the specified wavelength by ELISA reader. To do this test, after counting, 2×10^5cells were cultured in RPMI 1640 with 10% FBS, 100 IU/mL penicillin, 100 mg/mL streptomycin, and 2 mM L-glutamine in the absence and then the presence of phytohaemagglutinin (PHA) (GIBCO) as a mitogen. Cells were incubated at 37 °C with 5% carbon dioxide (CO_2) for 48 h. After removing of the supernatant and adding fresh culture medium, the cell suspension was incubated with 20 μL of MTT reagent (5 mg/mL) for 4 h at 37 °C with 5% CO_2. The plate was centrifuged at 2000 rpm for 10 min. The supernatant was removed, and 100 μL of isopropanol-HCl was added, and the absorbance was measured at 570 nm by an enzyme-linked immunosorbent assay (ELISA) plate reader (ICN Flow Titertek).

2.7. Statistical analysis

The obtained data were presented as the mean ± standard deviation for normally distributed variables, and median and quartiles for non-normally distributed variables. Hematological and pulmonary function test parameters were compared with *t*-test and their level with the Chi-square test. The comparison between the control and SM-exposed groups as well as between diseases subgroups were done via non-parametric Mann-Whitney test. The correlation between the viability of blood cells with qualitative variables was computed using the

Table 1
The demographic characteristics of study participants in control and SM-exposed groups (mean ± SD).

	Control group N = 109	SM-exposed group N = 110	*P* value
Age (years)	42 ± 7.6	50 ± 6.4	< **0.001**
BMI (kg/m^2)	28 ± 4	25.9 ± 5	**0.003**
Co-morbidity diseases			
Cardiac diseases (N[%])	0	30 (28.6%)	–
Kidney diseases (N[%])	0	26 (24.8%)	–
Liver diseases (N[%])	0	16 (15.1%)	–
Gastrointestinal diseases (N[%])	0	60 (56.6%)	–
Psychiatric diseases (N[%])	0	74 (71.8%)	

Comparison between two groups was performed by Student's t-test. Bold numbers show significant differences with *P*-value ≤0.05. SM: sulfur mustard; BMI: body mass index.

Spearman rank correlation coefficient.

Data analyses were done in SPSS version 23 (IBM Co., Redmond, NY). *P* values < 0.05 were considered significant.

3. Result

3.1. Analysis of the study population

Although all participants were in the age range of 35–60 year, the mean ± SD age of the SM-exposed patients was significantly higher than that of the control group (50 ± 6.4 vs. 42 ± 7.6, $P < 0.001$). In addition, the mean ± SD body mass index(Kg/m^2) in the SM-exposed patients was significantly lower than that in the control group (25.9 ± 5 vs. 28 ± 4, $P = 0.003$). According to clinical examination, some of the SM-exposed patients suffered from co-morbidities, including cardiac disease (28.6%), kidney disease (24.8%), liver disease (15.1%), gastrointestinal disease (56.6%), psychiatric disease (71.8%), and allergic disease (5.7%) (Table 1).

In this study, pulmonary function was assessed by spirometry test. As shown in Table 2, spirometry parameters revealed that in the SM-exposed group, the level of forced expiratory volume in 1 s (FEV1), forced volume vital capacity (FVC), and their ratio, were significantly lower than those in the control group ($P < 0.001$). Moreover, most SM-exposed people had abnormal pulmonary auscultation.

The severity of pulmonary complications in the SM-exposed subjects was classified based on Iranian Medical Committee of the Foundation of Martyrs and Veterans Affairs. This categorization is based on pulmonary auscultation and spirometry findings. So, 11.8% of the SM-exposed patients were healthy, 30.6% and 57.6% of them had mild/moderate and severe/very severe pulmonary complications, respectively (Table 3).

Table 2
Comparison of pulmonary function test between control and SM-exposed groups (mean ± SD).

	Control group	SM exposed group	*P* value
Pulmonary function test			
FVC (% predicted)	90.33 ± 13.09	57.69 ± 21.17	< **0.001**
FEV_1 (% predicted)	97.47 ± 15.43	54.43 ± 26.2	< **0.001**
FEV_1/FVC (% measured)	85.86 ± 5.68	69.81 ± 15.87	< **0.001**
Abnormal pulmonary auscultation (%)	0	88.9%	–

Comparison between two groups was performed by Mann-Whitney test. Bold numbers show significant differences with *P* value ≤0.05. SM: sulfur mustard; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s; FEV_1/FVC: the ratio of FEV_1 to FVC expressed as a fraction.

Table 3
Classification of pulmonary involvement severity based on pulmonary assessment in SM exposed group.

Pulmonary assessment	Count	Percentage
Normal	10	11.8%
Mild & moderate	26	30.6%
Sever & very severe	49	57.6%

The classification of severity of pulmonary involvement in SM exposed patients according to clinical assessment using criteria verified by the Iranian Medical Committee of the Foundation of Martyr and Veterans Affairs.

Table 4
Comparison of PBMCs viability between control and SM-exposed groups (mean ± SD).

	Control group	SM exposed group	*P* value
Viability in stimulated cells (%)	0.32 ± 0.1	0.29 ± 0.12	**0.016**
Viability in unstimulated cells (%)	0.29 ± 0.11	0.27 ± 0.11	0.125
Stimulation index (%)	114.62 ± 22.68	111.83 ± 20.71	0.239

Comparison between two groups was performed by Mann-Whitney test. Bold numbers show significant differences with *P* value ≤0.05. Stimulation index: A1-A0/A1*100. A = Absorbance (OD).

3.2. The vital activity of PBMCs in SM-exposed patients with long-term pulmonary complications

Based on statistical analysis, as shown in Table 4, the vital activity of PBMCs in the SM-exposed group, in the presence of PHA, was significantly lower than that in the control group (0.29 ± 0.12 vs. 0.32 ± 0.1, $P = 0.016$). Whereas, there was no significant difference between the two groups in the viability of PBMCs, in the absence of PHA. The stimulation index (percentage of surviving cells) of PBMCs in the SM-exposed group and control group were 111.83 ± 20.71 and 114.62 ± 22.68, respectively, and there was no significant difference between the SM-exposed group and the control group.

3.3. Association between comorbidities and the vital activity of PBMCs in the SM-exposed patients with long-term pulmonary complications

According to clinical examinations of specialists, some of the SM-exposed patients suffered from other diseases. Therefore, the SM-exposed group was classified based on having or not having comorbidities and compared with themselves and the control group. As shown in Fig. 1, the SM-exposed patients with cardio-vascular, kidney and gastrointestinal diseases exhibited significantly lower levels of the vital activity of PBMCs in the presence of PHA compared with the control individuals, and this level was significantly lower in individuals with/without liver and psychiatric disease compared to the control group. It is worth noted that there was no significant difference between comorbidities and vital activity of PBMCs in the absence of PHA.

3.4. Association between the vital activity of PBMCs and the severity of pulmonary problems in the SM-exposed patients with long-term pulmonary complications

In this work, as shown in Table 5, the vital activity of PBMCs in the SM-exposed individuals with abnormal auscultation, in the presence of PHA, was significantly lower than that in the control group (0.29 ± 0.13 vs. 0.32 ± 0.1, $P = 0.015$). Also, the association between pulmonary assessment and vital activity of PBMCs, in the presence of PHA, was analyzed. There was a significantly lower level of vital activity of PBMCs in severe and very severe SM-exposed patients compared to the control group.

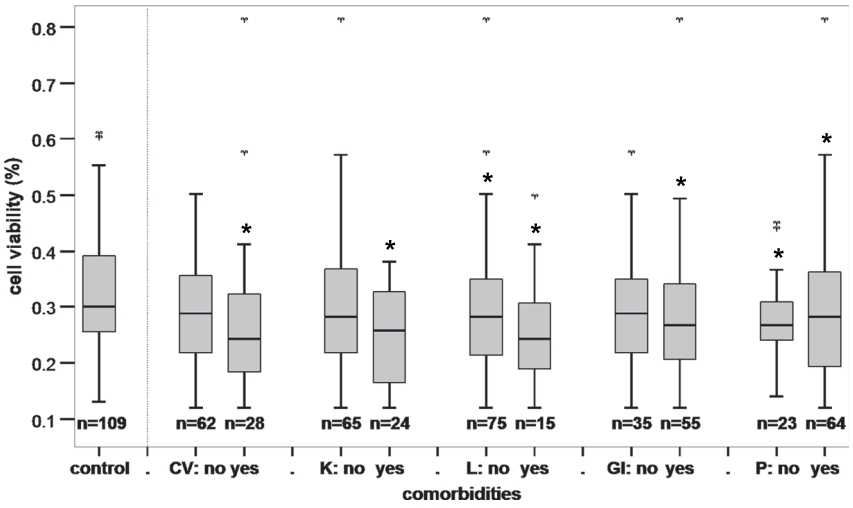

Fig. 1. Comparison of PBMCs viability in the presence of PHA with comorbidities between control and SM-exposed groups.
Statistical analysis was performed by Mann-Whitney test. The SM-exposed individuals with cardio-vascular, kidney and gastrointestinal diseases showed significantly lower levels of the vital activity of PBMCs in the presence of PHA comparing with the control group, and this level was significantly lower in individuals with/without liver and psychiatric disease comparing with the control group.
The star (*) shows significant differences with P value ≤ 0.05.
The ϒ presented outliers (> 1.5 interquartile range).
P = P-values.
CV: cardio-vascular diseases. K: kidney diseases. L: liver diseases. GI: gastro-intestinal diseases. P: psychological diseases. Yes: exposed patients who had disease, No: exposed patients who did not have disease.

3.5. Association between the vital activity of PBMCs with spirometry finding in the SM-exposed patients with long-term pulmonary complications

According to statistical analysis shown in Table 6, there was no significant correlation between the vital activity of PBMCs and spirometry findings like FVC%, FEV1, and FEV1/FVC (%) in the SM-exposed patients.

3.6. The hematological parameters in SM-exposed patients with long-term pulmonary complications

The results of the current study indicated that in the SM-exposed group, the total count of WBC and neutrophil were significantly higher than those in the control group but the lymphocyte count was significantly lower than that in the control group. The SM-exposed group had a significantly higher MCV and HCT percentage and lower MCHC than the control group. There was no significant alteration in other hematological parameters in both groups (Table 7).From a clinical point of view, there were no significant differences in the amount of HCT and MCV between study groups, whereas there were significant differences between the control and SM-exposed group in the amount of WBC, MCHC, neutrophil, and lymphocyte counts (Table 8).

4. Discussion

After more than two decades of SM intoxication, its long-term pulmonary complications are still the most critical problems and main health concerns in SM-exposed people. Given the importance of bloodstream in transferring the absorbed SM to other tissues [15], the present study was conducted to investigate the cytotoxic effect of SM on the vital activity of PBMCs and hematological parameters in chemical veterans with long-term pulmonary complications 25 years after SM exposure.

The first finding of the present study showed that in the presence of PHA, the vital activity of PBMCs in SM-exposed individuals was significantly lower than that in the control group. Also, in SM-exposed individuals with abnormal auscultation and severe or very severe pulmonary complications, the vital activity of PBMCs was significantly lower than that in the control group. In MTT reduction, as a cell viability assay, conversion of MTT tetrazolium into purple colored formazan, has occurred in viable cells with active mitochondria. It should be considered that the amount of signal generated due to MTT reduction is dependent on the either increasing of proliferation or activity of vital cells [21]. The reduction, obviously, might be due to a decreasing in cells activity or proliferation in SM-exposed patients compare with control group. Gould et al. showed that 2-chloroethyl ethyl sulfide intoxication (as an SM analog), produced mitochondrial dysfunction followed by an increase in mitochondrial reactive oxygen species (ROS) production and cellular oxidative stress [22]. In view of the present finding, the marked decline in the cell viability of the SM-exposed patients may be due to the oxidative damage in mitochondria, DNA and protein alkylation of mitochondria, and diminished mitochondrial activity induced by SM in these patients. To our knowledge, there is no comparable study to date, to demonstrate the effect of SM on cell viability in SM-exposed patients. There are, as of now, only a few in vitro studies on the effects of SM on cell viability [23–26].

Table 5
Association of PBMCs viability in the presence of PHA severity of pulmonary complications and pulmonary Auscultation in SM-exposed patients.

	PBMCs viability							
	N	Median	Q1	Q3	Mean	SD	P value[1]	P value[2]	P value[3]
Control	109	0.3	0.26	0.39	0.32	0.1			
Pulmonary assessment									
Normal	9	0.29	0.22	0.35	0.28	0.09	**0.341**		
Mild& moderate	23	0.24	0.19	0.38	0.29	0.11	**0.095**	0.850	
Severe& very severe	41	0.26	0.19	0.34	0.29	0.15	**0.007**	0.553	0.690
Auscultation									
Normal	16	0.29	0.23	0.38	0.31	0.1	0.567		
Abnormal	45	0.27	0.2	0.34	0.29	0.13	**0.015**	0.294	

The percentage of cell viability in control and SM-exposed groups at different severity stages of pulmonary complications and pulmonary auscultation were presented as median with first (Q1) and third (Q3) quartile and also mean ± SD. P value[1]: comparison with control group (Mann-Whitney). P value[2]: comparison of normal lung with pulmonary complications patients in SM exposed group (Mann-Whitney). P-value[3]: Comparison of mild & moderate with severe& very severe pulmonary complication groups in SM-exposed (Mann-Whitney). Bold numbers show significant differences with P value ≤ 0.05.

Table 6
Correlation between spirometry findings and cell viability in SM-exposed patients and control individuals.

		Control group		SM-exposed group	
		Stimulated cells	Unstimulated cells	Stimulated cells	Unstimulated cells
FVC%	r	**−0.252**	−0.187	0.093	0.158
	P-value	**0.025**	0.100	0.482	0.227
FEV1%	r	**−0.286**	**−0.232**	0.037	0.203
	P-value	**0.010**	**0.040**	0.775	0.114
FEV1/FVC (%)	r	−0.042	−0.058	0.090	0.092
	P-value	0.714	0.613	0.537	0.529

SM: sulfur mustard; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s. r: Spearman's rank correlations. Bold data shows significant differences with *P* value ≤0.05.

Table 7
Comparison of hematological parameters between control and SM-exposed groups (mean ± SD).

	Control group N = 109	SM-exposed group N = 110	*P*-value
WBC $10^3/mm^3$	6894.5 ± 1313.2	9677 ± 13,582.9	**0.030**
Neutrophil %	51.9 ± 7.3	62 ± 11.7	< **0.001**
Lymphocyte %	38.3 ± 6.6	29.6 ± 11.1	< **0.001**
Eosinophil %	5.5 ± 7.2	4.8 ± 6.9	0.820
MIX (EOS.MON.BAND)%	9.8 ± 2.5	9.2 ± 2.8	0.100
RBC $10^6/mm^3$	5.3 ± 0.4	5.4 ± 0.7	0.140
Hb g/dL	14.9 ± 1.0	15 ± 1.8	0.790
HCT%	43.5 ± 2.9	45.5 ± 4.9	< **0.001**
Plat $10^3/mm^3$	23.2 ± 5.9	24.5 ± 6.3	0.110
MCV fl	82.3 ± 3.3	84.6 ± 6.3	< **0.001**
MCH pg	28.2 ± 1.3	27.8 ± 2.7	0.180
MCHC %	34.3 ± 1	32.9 ± 1.5	< **0.001**

SM: sulfur mustard; *P* value: comparison with control group (*t*-test). Bold data shows significant differences with *P* value ≤0.05.

Table 8
Comparison of clinical levels of hematological parameters between control and SM-exposed groups.

		Control group N = 109	SM-exposed group N = 110	*P*-value
WBC	< 4500	1 (0.9%)	0 (0.0%)	**0.006**
$10^3/mm^3$	4500–11,000	107 (98.2%)	102 (89.5%)	
	> 11,000	1 (0.9%)	12 (10.5%)	
RBC	< 4.5	3 (2.8%)	8 (7.0%)	**0.012**
$10^6/mm^3$	4.5–6	103 (94.5%)	93 (81.6%)	
	> 6	3 (2.8%)	13 (11.4%)	
Hemoglobin	< 13.5	9 (8.3%)	21 (18.4%)	**0.029**
g/dL	13.5–18	100 (91.7%)	91 (79.8%)	
	> 18	0 (0.0%)	2 (1.8%)	
Hematocrit	< 40	10 (9.2%)	13 (11.4%)	0.193
%	40–54	99 (90.8%)	98 (86.0%)	
	> 54	0 (0.0%)	3 (2.6%)	
MCV	< 80	29 (26.6%)	15 (13.2%)	**0.012**
fl	80–96	80 (73.4%)	99 (86.8%)	
	> 96	0 (0.0%)	0 (0.0%)	
MCH	< 27	18 (16.5%)	22 (19.3%)	0.191
pg	27–31	91 (83.5%)	89 (78.1%)	
	> 31	0 (0.0%)	3 (2.6%)	
MCHC	< 32	1 (0.9%)	29 (25.4%)	< **0.001**
%	32–36	103 (94.5%)	85 (74.6%)	
	> 36	5 (4.6%)	0 (0.0%)	
Platelet	< 130,000	0 (0.0%)	1 (0.9%)	0.368
$10^3/mm^3$	130,000–450,000	108 (99.1%)	113 (99.1%)	
	> 450,000	1 (0.9%)	0 (0.0%)	
Neutrophil	< 55	71 (65.1%)	29 (25.4%)	< **0.001**
%	55–74	38 (34.9%)	74 (64.9%)	
	> 74	0 (0.0%)	11 (9.6%)	
Lymphocyte	< 20	1 (0.9%)	14 (12.4%)	< **0.001**
%	20–40	62 (56.9%)	88 (77.9%)	
	> 40	46 (42.2%)	11 (9.7%)	

SM: sulfur mustard; *P* value: comparison with control group (chi square test). Bold data shows significant differences with *P* value ≤0.05.

As another result, hematological findings showed a significant increase in total WBC count in the SM-exposed patients, and differential WBC revealed that neutrophil count was significantly increased, however, lymphocyte count was significantly decreased in the SM-exposed patients. The increased total WBC count and decreased lymphocyte count in the SM-exposed patients could be associated with a high incidence of respiratory infectious disease and inflammation in these people.

The analysis of hematological parameters showed a significant increase in HCT and MCV but a significant decrease in MCHC in the SM-exposed patients. Hemoglobin (Hb) in red blood cells carries oxygen from the lungs throughout the body. Increased HCT indicates blood disorder, dehydration, lung diseases, or other medical problems. In these patients, a hypoxemic status due to pulmonary problems could be associated with a significant increase in HCT. Furthermore, the effects of SM on other hematological parameters like a significant increase in MCV and significant decrease of MCHC could indicate that SM, in a dose-dependent manner, by attacking the bone marrow, could cause aplastic anemia or ineffective hematopoiesis [27]. Other studies have shown that the early investigation on hematological parameters of the SM-exposed people demonstrated leukocytosis as a common finding in the first few days after SM-exposure. Then, on the third or fourth day, the condition is followed by leukopenia, lymphopenia, neutropenia, thrombocytopenia, and anemia as well as a severe decrease in cellularity of bone marrow [28,29]. Hematological data from chemical victims, 17–20 years after SM-exposure, indicate that total counts for WBC and RBC, as well as HCT, were significantly higher in these patients [3]. In the Sardasht–Iran Cohort Study (SICS), we evaluated the effect of SM on peripheral blood cells, 20 years after SM exposure and found that the patients had significantly higher HCT, MCH, MCV, lymphocyte count and in contrast, significantly lower PLT, MCHC, WBC, PMN values than the control group. These findings regarding the normal ranges indicate that all of the changes in hematological parameters are not clinically significant [30]. Khazdair et al. showed a significant increase in total WBC count, MCV, and HCT values but a significant decrease of monocytes and MCHC in the SM-exposed patients, 27–30 years after exposure. From a clinical point of view, all of them were in the normal range except for the neutrophil count [16]. In the current study, most of the changes in hematological variables are clinically significant. Differences between studies can be due to the differences in study groups, inclusion criteria, sample size, the severity of problems, intensity and frequency of exposures, and history of therapy.

5. Conclusion

According to the present findings, hematological complications in SM-exposed patients like increased total count of WBC, neutrophil, and

HCT percentage could be due to respiratory disorders and these complications might play an important role in increasing the risk of infectious diseases and other malignancies. For better understanding of the pathological effects of SM on blood cells and treatment of these patients, further studies like investigation on their bone marrow and hematopoietic stem cells are required.

It should be noted that the most important limitation of the present study was inaccessibility to a complete list of drugs used by participants.

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Declaration of competing interest

The authors report no conflict of interest in this study.

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