

# Serum levels of interleukin 8 and matrix metalloproteinase 9 levels in pulmonary chemical patients exposed to sulfur mustard

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## Abstract

**Background:** Chemical gases were one of the war instruments of Iraq against Iran that caused skin, eye, blood, heart, and kidney complications. We aimed to investigate interleukin (IL-8) and matrix metalloproteinase 9 (MMP-9) serum level in chemical lung patients of sulfur mustard and its relationship with lung complications. **Materials and Methods:** The present study was conducted on 40 patients who were exposed to mustard gas. To determine the serum level of IL-8 and MMP-9, 5 cc, blood samples were taken from subjects in a sterile condition. Then, spirometry test was performed on pulmonary patients to investigate the pulmonary function, and its effect on biomarkers was studied and their body mass index was performed before spirometry. Then, data were analyzed using statistical package for the social sciences 22 and descriptive and analytical tests. **Results:** Finding has shown that a significant difference exists in all blood factors in experimental and control groups. Correlation test did not show a significant relationship between blood factors and respiratory factors in chemical veterans. **Conclusion:** The present study had found a significant difference between IL-8 and MMP-9, and this requires more studies in the future.

**Key words:** Interleukin 8, Iran, matrix metalloproteinase 9, mustard gas

## INTRODUCTION

The use of chemical weapons can be considered as the inhumane catastrophe of the 20<sup>th</sup> century with uncontrollable effects and consequences.<sup>[1]</sup> However, the widest use of mustard gas (MS) is related to chemical attacks of Iraq against Iranian forces and Iraqi Kurds during 1983–1988.<sup>[2]</sup> It can be caused on skin, eye, blood, heart, lung, and kidney complications. Chemical gases in this war include different types, and some of them were used by Iraq for the 1<sup>st</sup> time.<sup>[3]</sup> MS is one of the most important poisonous gases that caused detrimental effects on the pulmonary system of veterans followed by wounds, inflammation, and spasm.<sup>[4-7]</sup>

One of the most common diseases of chemical veterans is chronic obstructive pulmonary disease.<sup>[8]</sup> This disease is referred to the diseases in which air flow is limited and is not completely reversible. These groups of diseases include chronic bronchitis and emphysema.<sup>[9]</sup> Chronic obstructive pulmonary diseases as a disability factor in activities, family

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roles, social context, and daily activities lead to a decreased quality of life in patients.<sup>[10]</sup>

In a study, in 2002, has shown that most common clinical symptoms of chemical gases including mustard were cyanosis (27.6%), crackles (23%), and wheezing (9.2%).<sup>[11]</sup> In a study by Rowell and colleagues, it was shown that exposure to MS, in addition to physical problems, leads to long-term social and economic problems with negative effects on veterans and their family members.<sup>[12]</sup>

According to various studies, no similar study on respiratory and blood problems of veterans was found. According to the importance of issues related to exposure to MS, this study was conducted to determine the relationship between serum levels of interleukin-8 (IL-8) and matrix metalloproteinase 9 (MMP 9) in pulmonary chemical patients exposed to MS and healthy people.

## MATERIALS AND METHODS

It was a cross-sectional study which has conducted on 40 patients exposed to MS in a specific war session about 30 years before that were living in Ahvaz city in 2016.

We were invited 40 patients who were willing to participate in this study which was selected based on convenient sampling method; five of them were excluded due to recurrence of disease and exacerbating conditions. Furthermore, 6 patients were excluded due to smoking, 6 patients were excluded due to cardiovascular, and coronary artery diseases, and one patient was excluded due to the history of cancer. Ultimately 22 patients, who were all exposed to an equal dose of SM at one war session and were now in stable phase, were identified as cases. None of these patients had a history of hospitalization or specific treatment or immunosuppressant within the past 3 months before the study and did not have any inflammatory disease or disease that changes the inflammatory markers. We also invited 22 healthy men in similar age, who were lived in the same weather conditions, were selected as the control group. All patients signed an informed consent voluntarily and completed a questionnaire containing the demographic data of patients. Blood sample was taken as 5 mL through the median vein of the right hand and transferred to the laboratory at -20°C, centrifuged at 3000 rpm for 5 min and kept at a temperature of -70°C. Spirometry of patients was performed in the standard sitting position with a nose strap and a minimum of three successful expirations, while they had not eaten and drunken for the past 3 h.

Spirometry test was performed on chemical veterans who were exposed to the MS to investigate the pulmonary function and its effect on blood biomarkers in standard sitting position with nose strap and a minimum of three successful expirations, while they had not drunken, eaten, and smoked for 3 h before the test. Furthermore, to determine body mass

index, the height of participants was measured in standard condition without shoes, and their weight was measured before spirometry. It should be noted that the spirometer device was calibrated before the test. Then, pulmonary experts controlled the test. Furthermore, lung hearing and respiratory symptoms including chronic cough, phlegm, shortness of breath, and chest pain were examined.

## Enzyme-linked Immunosorbent Assay (ELISA) Method

Serum obtained after centrifugation was tested by IL-8 and MMP9 ELISA kit manufactured by R and D company through sandwich ELISA method.

## Ethical Issue

The procedures of this study were approved by the Independence Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (IR.AJUMS.REC.1394.199), and written informed consent form was taken from all parents participate

## Statistical Analysis

Kolmogorov–Smirnov test was performed before statistical analysis to examine the normality of the variables. The results were presented in the form of statistical tables and numeric indicators. Chi-square test, correlation, and nonparametric test (Wilcoxon W) were used to analyze the data. Variable values were expressed as frequency, mean  $\pm$  standard deviation (SD). Statistical calculations were performed using (Statistical Package for the Social Sciences version 20 Inc., Chicago, IL, USA). For all statistical analyses,  $P < 0.05$  was considered as significant.

## RESULTS

The results show that the mean and SD was  $53.36 \pm 3.68$ . According to the results, no significant difference exists between demographic indexes (weight, height, and age) of veterans [Table 1].

The results of respiratory indexes mean and SD show that the mean of forced expiratory volume in 1 s (FEV1) that is the amount of output gas during the first second of excessive and forced exhaustion of the lungs was obtained as  $82.09 \pm 1168$ . Furthermore, this level for forced vital capacity that includes air volume is  $74.72 \pm 1252$ . Furthermore, for FEV1/FVC where the air can leave the lung in the first exhalation, this level was  $86.36 \pm 6.98$ , and for peak expiratory flow rate, mean and SD level was obtained as  $85.14 \pm 55.18$ . The results of Table 2 show that blood factors show a significant difference in research groups.

The results of the correlation test did not show a significant difference between IL-8, MMP9, and respiratory factor in chemical veterans Table 3.

## DISCUSSION

The greatest complications resulted from exposure to MS in organs such as disorder in ventilation system, airway secretions, airway bleeding, perfusion, lung biochemical disorders, lung vascular damage, and impaired lung defense systems.<sup>[13]</sup> The most common symptom that patients with mustard poisoning show is chronic bronchitis. This disease shows itself as a chronic cough, shortness of breath, and sputum with different degrees.<sup>[14]</sup> The present study aimed to investigate the relationship between serum levels of IL-8 and MMP 9 in pulmonary chemical patients with MS and healthy

people. The results showed that a significant difference exists between control and intervention groups in the levels of IL-8 and MMP 9. The reason is that MS in chronic phase can cause upregulation of inflammatory mediators such as IL-8 and other cytokines.<sup>[15]</sup> These results are consistent with Attaran *et al.* who showed that serum level of IL showed differences in different chemical patients who were exposed to mustard and the serum level of this marker was decreased in some patients.<sup>[16]</sup> Yaraee *et al.* showed that the serum level of this marker is increased.<sup>[17]</sup>

The results of a study by Gabrielsen and Good.<sup>[18]</sup> showed that MS has short-term and long-term effects on the immune system through antibodies and cellular immunity. In a study by Zandieh *et al.*, it was shown that long-term effects of MS on the immune system decrease cellular immune at the first 3 years after exposure.<sup>[19]</sup> Furthermore, in a study by Ghotbi *et al.*, it was shown that exposure to mustard in chemical veterans is significantly lower than the control group and this is consistent with the present study.<sup>[20]</sup>

## CONCLUSION

This study was the first study in Iran that was conducted on these subjects, and no similar study was available. In a general conclusion, a significant difference was observed between the findings of blood factors in research groups and total blood factors, particularly IL-4 and metalloproteinase 9, in veterans exposed to mustard. However, the results of correlation test did not show a significant difference between respiratory and blood factors. Since MMP 9 has been reported as a marker gene for most of the cancers, it is suggested to conduct a study on IL-4 and MMP9.

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## Conflicts of Interest

There are no conflicts of interest in this research.

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**Table 1: Anthropometric indices and demographic of subjects**

Title	Group	Mean±SD	P
Age	Case	53.36±3.68	0.12
	Control	56.77±9.38	
Weight	Case	79.84±14.00	0.59
	Control	81.88±10.69	
Height	Case	169.76±6.34	0.76
	Control	170.27±4.63	
BMI	Case	27.92±4.60	0.70
	Control	28.41±3.60	

BMI: Body mass index

**Table 2: Comparing the concentration of blood factors in research groups**

Title	Groups	Mean±SD	P-value
MMP9	Case	0.79±0.33	0.04
	Control	0.61±0.19	
IL-8	Case	1.15±0.54	0.001
	Control	0.68±0.07	

IL 8: Interleukin-8

**Table 3: Correlation between serum levels of IL-8, MMP9, and PFTs**

Variable	FEV1	FEV1/FVC	PEFR
IL-8			
Correlation coefficient	0.16	0.01	0.26
P value	0.48	0.95	0.25
MMP 9			
Correlation coefficient	0.33	0.09	0.07
P value	0.13	0.7	0.75

IL-8: Interleukin-8, PEFR: Peak expiratory flow rate, FEV1: Forced expiratory volume in 1 s, FVC: Forced vital capacity

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