Effects of mustard gas on immune system of exposed Iranian people: a review of conducted studies

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ABSTRACT

Exposure to high dosages of sulfur mustard (SM) can cause bone marrow depression, immune system suppression, impairment of the immune functions, and eventually results in diseases due to secondary immune disorders. In this article, we have studied the effects of this poison on Iranian veterans by analysis of related published studies. In a systematic search, the effects of SM gas on Iranian victims were reviewed. We used known international medical databases such as ISI, Medline, Scopus and Iranian databases such as Iranmedex and Irandoc. About 350 published articles were assessed. Among them, 43 articles were related to immunologic field. No special evaluation was conducted on the quality of the reviewed manuscripts and the credit of journal was considered sufficient. In accomplished studies conducted on Iranian people, both cellular and humoral immunity were affected. The reported changes were as follows: increasing the number of inflammatory cells in chronic phase which indicates ongoing active alveolitis, neutrophils (in chronic bronchitis), eosinophils, CRP titer, RF titer, IgG (especially in asthmatic patterns) ,IgM , Ig E, IL-6, TFG-beta1target protein in bronchoalveolar lavage fluid, and decreasing the number of leukocytes , lymphocytes, natural killer cells (NKCs), IL-8and IL-6 in blood. Eventually, in reported changes, chemo taxis factors, plasma opsonins and nitroblue tetrazolium (NBT) test were normal. In sever and prolonged exposure to mustard gas, the immune system would be suppressed. Therefore, the victims should be monitored for infections and even cancers.

Keywords: Mustard Gas; Immune system; Cellular immunity; Humoral immunity.

INTRODUCTION

During Iraq-Iran war (1980-1988), Iranian people 387 times were exposed to chemical attacks with Iraqi artilleries and rockets. In this war, more than 1,000 tons of mustard gas was poured over Iranian people [1]. Iranian researchers have carried out a lot of researches on the effects of SM on the body of exposed people [2-5]. One area of accomplished researches is the effects of this poison on the human's immune system.

Exposure to SM, affect both cellular and humoral immunity [6] and high levels of this poison can cause systemic poisoning, and subsequently suppress the activity of bone marrow (bone marrow depression) and eventually develop immune system suppression [7-8]. Thus, SM, can impair the immune function and lead to diseases due to secondary immune disorders [9]. This poison can also cause opportunistic infections, septicemia, and even death among sever immunosuppressed patients [10]. In humans, exposure to low doses of sulfur mustard, will damage the cellular immunity, but the humoeral system and production of antibodies, not only did not impair, but even in some cases will be more active, while in exposure to high doses of this agent, both cellular and humoeral immunity will be damaged [9]. Sulfur mustard is also effective on complement system [7]. Mahmoudi et al investigated 40 male subjects who had severe clinical complications 16 to 20 years after exposure in Khorasan province (in the east of Iran). In this study, the total counts of White Blood Cells (WBCs), monocytes percentages, IgM , C3, alpha(1&2) and beta globulin levels were significantly higher in the patients than the controls. They concluded "sulfur mustard can cause long-term effect on the immune system of the patients with severe poisoning. The impaired immunity is probably responsible for

increased risk of infections in exposed patients" [11]. Hefazi et al studied late complications of SM on skin and immune system 16 – 20 years after exposure. There did not find any correlation between the severity of late cutaneous lesions and immunological complications [12].

In this article, we studied the effects of this poison on immune system of Iranian people exposed to sulfur mustard gas by analysis of accomplished studies.

MATERIALS AND METHODS

In a systematic search, the effects of sulfur mustard gas on Iranian victims were reviewed. We used known international medical databases such as ISI, Medline, Scopus and Iranian databases such as Iranmedex and Irandoc. About 350 published articles were assessed. Among them, 43 articles were related to immunologic field (35 papers were published by Iranian authors and 8 papers were published by the others). The main criterion for qualification and selection of the manuscripts was their publication in approved medical journals.

RESULTS

Effects on peripheral blood cells:

First after exposure, leukocytosis and lymphopenia will be occurred, then, it will be returned to normal ranges within 4 weeks in recovered exposed people. Among the fatal cases, due to bone marrow depletion, marked cytopenia (leukopenia, lymphopenia, neutropenia) will be occurred. Neutropenia will increase susceptibility of the patients to secondary infections [13].

According to Emad et al study, in patients with pulmonary fibrosis who were exposed to SM, the number of neutrophils and eosinophils was significantly higher than the control group [14].

Effects on phagocytic activity:

In Bahar's and colleagues' study have stated that the phagocytic activity of the exposed patients to SM, within one month after exposure show a sharp decline. So that, in some cases the phagocytic index reduced to 1/5 of normal range, whereas the opsonin index do not much decrease and its condition is better than phagocytic index. These changes will return to normal status after 3 months [15]. Keyhani and colleagues on 121 Iranian casualties who had contaminated with sulfur mustard, using qualitative NBT test, showed that the function of neutrophils in a period up to 51 days after exposure were positive in all of cases. This finding might indicate lack of neutrophil dysfunction [16].

Zandieh and colleagues had also assessed cell movement, chemotactic factors, and plasma and cell opsonins and the results were normal [17].

Impaired cellular immunity:

One of the main assumptions is that SM has a detrimental effect on cellular immunity [11] and T-cells are decreased [1].

Emad A and Emad Y found that CD8 T cells in Bronco Alveolar Lavage (BAL) fluid were significantly elevated in patients with pulmonary fibrosis [19]. Researches have shown that mustard gas by making numerous changes in lymphocytes can reduce their activities. These changes included loss of microvilli, the appearance of large vacuoles in the cytoplasm, loss of cytoplasmic components, chromatin condensation in cellular nuclei, and a lot of holes in the lymphocytes. According to published reports of chemical defense research institute affiliated with America's Army, lymphocytes will die about 4 hours after exposure to SM, [19]. Mohammad hoseini akbari et al conducted a study on 113 Iranian veterans 25 years after poisoning. They showed that severe exposure to SM might cause long-term damages to the immune system in veterans. The total count of white blood cells (WBCs) were significantly higher in SM-exposed people than control subjects (P=0.008). As well as, "the percentages of total CD4+ lymphocytes were significantly lower in sulfur-mustard-exposed veterans than they were in control subjects (P<0.001)" [20].

Shaker and colleagues in another study which conducted on 20 healthy and 75 exposed men, examined helper and cytotoxic T cells 10 years after exposure. They divided the patients in to three groups (mild, moderate and severe) according to their level of contamination. They used four monoclonal antibody markers for evaluation of the number of T helpers (CD3+/CD4+) and T cytotoxic (CD8+). They applied anti CD3+and anti CD4+ for the evaluation of T helpers, anti CD8+ for the evaluation of cytotoxic T cells, anti CD45+ (a leukocyte antigen that is present in 90% of leukocytes) for evaluation of total leucocytes,

anti CD56(a marker of NKCs which existed in 70% of these cells and CD25+ (These are also existed in NKCs for evaluation of NKCs) [1]. In this study, the percent of CD45+ was normal in all groups, helper and cytotoxic T cells were significantly decreased in severe cases in comparison to mild one, and CD4 +/ CD25+ in severe cases were significantly higher than the other groups [21].

Ghazanfari et al reported that the percentages of CD45+/CD3+, CD45+/CD3+/CD4+, in exposed cases were significantly decreased and CD3+/CD16+56+ were increased [22].

CD14, CD16 and HLA-DR, are three markers that we can evaluate the functions of monocytes by them. CD14 is a specific marker of monocytes, CD16 has been known as a marker of stimulated cells, and HLA-DR has been identified as a marker for activated cells. Decreasing of HLA-DR in monocytes, increase the risk of infections [15]. Pourkaveh and colleagues in a case-control study conducted on 75 veterans (cases) and 10 healthy volunteers (controls) had examined the function of monocytes by above markers and concluded that there was no problem in monocyte cells in bone marrow but, the function of monocytes was not complete [23].

Nick siyar et al in a case-control study conducted on 237 veterans (cases) and 202 not exposed and healthy subjects (controls) had examined blood samples for investigating the large granular lymphocytes (LGLs), atypical lymphocytes, and hand mirror lymphocytes (HMLs). In this study, average number of leukocytes, neutrophils, eosinophills, hypersegmented eosinophis, monocytes, atypical lymphocytes, HMLs, LGLs, percentage pelgeroid polymorphs (PPPs) and G-score, among the exposed people to SM, was significantly greater than the healthy controls (not exposed people), but the difference between the mean platelet count and normal peripheral lymphocytes was not significant [24].

Effects on Natural Killer Cells:

Decreasing NKCs is the main cause of recurrent viral infections, septicemia and a high incidence of malignancies in patients exposed to SM [25]. There are contradictory results in NKCs counts in conducted studies. Some studies have shown that among workers who work in factories producing chemical weapons, the number of cytotoxic T cells are increased and the ratio of helper to T cytotoxic cells, as well as, natural killer cells are decreased [18]. Ghazanfari et al, in their study conducted on 372 SM exposed veterans and 128 non exposed people with the same ethnicity, culture, and demographic conditions, found that the count of NKCs in peripheral blood of exposed cases (particularly in SM exposed group with pulmonary problems) 20 years after the exposure was highly increased. They concluded that NKCs probably have a role in pathogenesis of pulmonary complications induced by SM [22].

Ghotbi and Hassan in one case-control study conducted on 75 severe pulmonary complicated patients who were injured with SM 10 years ago and 20 healthy volunteer have examined the natural killer cells, CD45+ / CD56+ by fellowcytometric analysis. They observed that in severe pulmonary cases the percentage of NKCs was significantly reduced compared to control group. The authors have also reported that CD8+/CD56+ T cells were in the normal range [20].

Effects on Cytokines:

Interleukin (IL)-8 is a potent neutrophil chemotactic factor and it is a definitive mediator in neutrophil-dependent acute inflammation. Various types of cells can produce IL-8, either in response to various stimuli or constitutively after malignant transformation. IL-8 has also a potential effect in viral infections and tumor progression [27].

Inflammatory mediators especially IL-8 and IL-6 play the primary role in the various chronic pulmonary diseases [28].

In one study accomplished by Ghasemi et al, serum IL-8 levels in all exposed people were significantly lower than the matched controls (P=0.002). Tear IL-8 levels in the selected exposed were significantly lower than in the selected controls (P=0.030) [29].

The levels of IL-6 and IL-8 in serum are significantly decreased in the SM exposed compared to the control group [28].

Attaran et al had evaluated serum levels of IL-6 in 50 poisoned patients with SM and stable COPD. They found that serum IL-6 is increased in patients with SM poisoning and COPD, and might have a direct association with airflow limitation [30].

Pourfarzam and colleagues in a cohort study on 348 cases and 120 controls who had been exposed 20 years ago to SM in Sardasht (a city in the west of Iran) had measured the serum CRP, IL-6, IL-8 and RF in patient with different

severity of lung diseases. To determine the severity of the lung disease, they used spirometry with criteria suggested by Thoracic American Association. Pourfarzam et al observed that the levels of IL-6 and IL-8 in exposed people compared to the control group were decreased significantly. They did not find any significant association between the level of serum IL-8 and clinical symptoms (chronic cough, sputum production, shortness of breath, hemoptysis) and pulmonary signs (crackles, rales, wheezing and spirometric parameters but IL-6 with wheezing and CRP with rales and wheezing were correlated. Pourfarzam and colleagues had concluded that although after exposure to SM the level of interleukin 8 and 6 were reduced, these inflammatory mediators did not play a major role in pathogenesis, stability of respiratory complications and severity of lung problems [28].

In a cohort study in Sardasht, Yaraee et al examined "the changes in serum levels of inflammatory cytokines (TNF, IL-1alpha, IL-1beta and IL-1Ra) 20 years after exposure to SM". They found that serum pro-inflammatory cytokine levels were significantly lower in the exposed group than in controls ($p<0.01$) [34].

Emad A and Emad Y demonstrated a significant correlation between CCL5, CCL11, and IL-5 levels as well as eosinophils in broncoalveolar lavage (BAL) of the patients with pulmonary fibrosis induced by SM gas inhalation. These findings suggest that the C-C chemokines (CCL5, CCL11) and IL-5 contribute to the recruitment of eosinophils in the lung of exposed victims [31].

Acute phase reactants:

There have been shown that increasing of CRP is an indicator of severity of diseases and mortality, and it can be expressed as a biomarker of systemic inflammation in COPD and also a high titer of RF can indicate the severity of pulmonary dysfunction [28].

Effects on humeoral immunity:

Studies conducted on animals reported that alkylating agents such as SM had a major effect on B lymphocytes suppression. So, hypogammaglobulinemia is a significant finding in animals [18]. Ahmadi in a study on five injured chemical victims had examined the effects of SM on humeoal immune responses and the secretion of immunoglobulins. This researcher concluded that in the first 24 hours and the second week after injury, no changes in antibody producing from B cells were observed. But in the second month, immunoglobulin levels were reduced compared with the control group [18].

In Keyhani et al's study, the serum levels of IgG, IgA and IgM of patients exposed to SM in the battlefield were measured by single radial immunodiffusion from third day up to one month after exposure. The serum levels of IgG in patients showed significant reduction on third day after exposure. However, the levels of IgG in the serum samples collected from the patients during 4-18 days after exposure to SM were found to increase. The increase in serum IgG levels in the sera of patients which were collected during 19-31 days after exposure to SM was found to be highly significant, surpassing those from the controls. The levels of serum IgA in patients during one month after exposure to SM showed alterations similar to those of serum IgG. However, the serum alterations of the patients IgA, comparing to those of the normal controls, were not significant. The serum levels of IgM in patients did not show marked alterations during one month after exposure to SM comparing to those of the normal controls. The initial decrease in serum levels of IgG in patients was discussed in terms of a possible leakage of IgG into the skin blisters and into other severely affected parts of the body such as respiratory system, whereas the subsequent increase in serum IgG was interpreted as due to (auto) antigenic stimulation of the patients' immune systems [32]. About the antibody changes, reports are contradictory. For example, according to one study, IgG, IgM and IgE among chemical injured people in comparison with the control group were increased [25]. It was found that among IgG subclasses (IgG1, IgG2, IgG3 and IgG4) and other immunoglobulin classes, only IgM and IgG4 were significantly decreased in serum of exposed cases [33]. We have presented the immunologic changes in people who were exposed to SM in tables 1-3.

Table1: Immunological changes in various conditions after exposure to mustard gas among Iranian victims after Iraq – Iran war (1980 – 1988).

Indicator	Iranian reports	Conditions	Reference
Total WBCs	Decreased	In early exposure	13
	Increased	20 years after exp.	11,20
	Pan Cytopenia (leukopenia, lymphopenia, neutropenia)	In fatal cases	13
Neutrophils	Increased	in pulmonary fibrosis	14
Eosinophils	Increased	in pulmonary fibrosis	14
Monocytes	percentages	severe clinical complications 16 to 20 years after exposure	11
	Functional disorders	in bone marrow	23
T Lymphocytes	Decreased		1,13
T- Helpers (CD3+/CD4+)	Decreased	in severe cases	21
T – Cytotoxic (CD8+)	Decreased	in severe cases	21
	Increased	In BAL fluid in pulmonary fibrosis	19
Natural Killer Cells (NKCs)	Decreased	Among workers who work by SM	22,26
	Increased	particularly in SM exposed group with pulmonary problems in late stage	14
Atypical lymphocytes (Hand Miror Lymphocytes, Pelgeroid Polymorphs)	Increased	In peripheral blood cells in exposed people	24
Neutrophilic G-Score	Increased		24
CRP	Increased	In sever and fatal COPD	28
RF	Increased (high titers)	indicate the severity of pulmonary dysfunction	28

changes in various conditions after exposure to mustard gas among Iranian victims after Iraq – Iran war (1980 – 1988).

Indicator	Iranian reports	Conditions	Reference
TNF	Decreased	20 years after exposure to SM	34
IL-1alpha			
IL-1beta			
IL-1Ra			
Interleukin 6	Increased	In COPD in exposed people	30
Interleukin 8	Decreased	In late stage, especially in chronic pulmonary diseases and tear of exposed people	28,29
CCL5 , CCL11 and IL-5	significant correlation	In BAL of the patients with pulmonary fibrosis	31

Table3: Changes of Immunoglobulin classes in various conditions after exposure to mustard gas among Iranian victims after Iraq – Iran war (1980 – 1988).

Indicator	Iranian reports	Conditions	Reference
IgM	Increased	severe clinical complications 16 to 20 years after exposure	11
IgG	decreased	On day 3 after exposure to MG	32
	Increased	one month after exposure to MG	32
IgE	Increased		25
IgA	Increased	one month after exposure to MG	32
alpha(1&2) and beta globulin	Increased	severe clinical complications 16 to 20 years after exposure	11

DISCUSSION

Researches have shown that mustard gas by making numerous changes in lymphocytes can reduce the activities in these cells [18]. In Iranian reports, among chemical veterans, even 10 years after exposure, immune system may be still impaired and this can be due to various health problems among them [10]. When the number of natural killer cells is decreased, the risk of cancers and infections will increase [18,25].

Iranian researchers stated that the marked reduction of NKCs in severe forms of pulmonary diseases induced by SM was probably due to destructive effects of this substance on precursor cells of NKCs in the bone marrow. They also reported that reducing the number of NKCs in severe cases, in addition to increasing the risk of cancers lead to a greater risk of viral infections [7,35]. However, some studies do not confirm these results. Hooshiar et al in their study on 85 veterans examined the cellular immunity by assessing the markers CD15, CD16 and CD45 and testing NBT but they did not observe a significant difference between chemically injured and normal subjects [9]. However, in intense exposures to SM, occurrence of cancers and infections must be considered.

Active T-helpers enhance the activity of specific and nonspecific components of the immune system. Furthermore, the cytotoxic T Cells have a major role in the fighting against viruses and tumors and reducing of these cells will cause various cancers including lymphoma and leukemia [21].

Shohrati et al did not show an increased incidence of cancers in Iranian victims in their study [36]. However, in other studies conducted on Iranian victims, nasopharyngeal carcinoma, bronchogenic carcinoma, gastric adenocarcinoma, as well as, myeloblastic and lymphoblastic leukemia were reported. In one study, the incidences of hematologic malignancies in Fars province were investigated and the incidence of acute myeloblastic leukemia and acute lymphoblastic leukemia were reported as 0.23% and 0.2% respectively. They were 18 times and 12 times higher than the control group. Also, Ghanei and colleagues had reported the appearance of chronic myeloid leukemia after the exposure to mustard gas [37-38]. There were some evidences about increased incidence of cancers among victims particularly lung cancers (probably in long term) [39]. Aditionally, Zafarghandi et al showed carcinogenesis following acute exposure to SM during the war [40] and stomach cancer, basal cell carcinoma, Bowen's carcinoma and spinocellular carcinoma [41].

On the basis of some researches, interleukin 8 is a chemokine which was produced by leukocytes (monocytes, T cells, neutrophils and NKCs) and the other cells such as endothelial cells, fibroblasts and epithelial cells. Some factors such as microbes and their products and environmental factors such as hypoxia can stimulate the production of IL-8 [28]. IL-8 plays an important role in the pathogenesis of chronic obstructive pulmonary disease(COPD) , pulmonary fibrosis and asthma. IL-8 has been suggested for detection of activity, severity and exacerbations of pulmonary diseases as a biomarker by some researchers [42-43]

Nocker et al investigated the level of IL-8 in airway secretions of patients suffered from asthma or COPD.

They found that the levels of this cytokine were increased in the airway secretions (bronchoalveolar lavage fluid) of the patients [42]. IL-6 is a cytokine that may also play an important role in the pathogenesis of COPD.

The amount of this substance in bronchoalveolar lavage fluid and exacerbation of COPD is raised [43]. Despite the above mentioned facts, Pourfarzam et al believed that interleukins 6 and 8 did not play a major role in pathogenesis, stability of respiratory complications and severity of lung problems [28]. In conclusion, in sever and prolonged exposure to mustard gas, the immune system would be suppressed. Therefore, these people should be monitored for infections and even cancers.

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