

## ORIGINAL ARTICLE

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# The Short-Term Effect of Mustard Gas on the Serum Immunoglobulin Levels

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## ABSTRACT

Mustard gas (MG), as a chemical warfare agent was used by the Iraqi army in Iran-Iraq conflict against military men in the battlefield in 1985.

The serum levels of IgG, IgA and IgM of patients exposed to MG in the battlefield were measured by single radial immunodiffusion from day 3 up to one month after exposure to MG.

The serum levels of IgG in patients showed significant decrease on day 3 after exposure to MG. However, the levels of IgG in the serum samples collected from the patients during 4-18 days after exposure to MG 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 MG was found to be highly significant, surpassing those from the controls. The levels of serum IgA in patients during one month after exposure to MG 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 MG comparing to those of the normal controls.

The initial decrease in serum levels of IgG in patients is 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 is interpreted as due to (auto) antigenic stimulation of the patients' immune systems.

**Key words:** IgG; IgA; IgM; Mustard Gas; Sulfur Mustard

## INTRODUCTION

Mustard gas was used as chemical warfare in Iran-Iraq conflict in 1985. The principal toxic component of the mustard gas is sulfur mustard. As an alkylating agent, sulfur mustard reacts with various molecules

altering their normal functions.

The harmful effects of sulfur mustard are due to its direct reaction with nuclei of cells, combination with amino acids arginine, cysteine, lysine and production of toxic compounds with various tissue metabolites.<sup>1,2</sup>

Studies of Hektoen and Corper<sup>3</sup> for the first time revealed the detrimental effects of mustard gas in the humoral immune system. This researcher showed that exposure of dogs and rabbits to mustard gas reduced specific antibody production in these animals. More recent studies indicated that sulfur mustard caused an

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overall suppression of the murine immune response to sheep red blood cells and delayed type hypersensitivity.<sup>4</sup>

Exposure of soldiers and civilians occurred during Iraq-Iran conflict. Human subjects who were exposed in the battlefield to mustard gas experienced variety of disorders. Initially the organs most severely affected were the skin, eyes and lungs. Blisters and vesicles were formed in various exposed parts of the skin.<sup>5</sup> Upon deeper penetration and absorption by organs and tissues, mustard gas induced alterations in the endocrine and immune system variables. The decrease in the numbers of the cells and molecules of the immune system led to immune suppression in the mustard gas victims, predisposing them to infections and malignancies.<sup>6, 7-9</sup>

As the results of the injuries caused by the mustard gas in the skin, eyes, lung and other organs, the mustard gas victims were very susceptible to develop various infections in particular by opportunistic microorganisms.<sup>10</sup> Determination of serum immunoglobulins in subjects shortly after exposure to mustard gas can reveal the most susceptible targets of this chemical warfare in the immune system. The findings of such investigation can also shed light on the strategies to neutralize the toxic effects of mustard gas in the immune system and the ways to protect the victims against infection. In the present study, we determined the serum immunoglobulin levels of the patients who had been exposed in the battlefield to mustard gas from day 3, up to one month after exposure.

## PATIENTS AND MEHTODS

**Patients and Controls-** the patients were soldiers, all male and mostly in their 3<sup>rd</sup> decade of their life who had been exposed to mustard gas in the battlefield in Iraq- Iran war during 1986. These victims had been transferred initially to a military hospital and very soon to a general hospital in Tehran for special treatment. Aliquots of the venous blood samples which had been collected from these patients for routine hospital investigation were also used for determination of serum levels of IgG, IgM and IgA. The sera which were used for this study were those collected from the patients during one month hospitalization. These sera consisted of those collected on day 3, during 4-18 days after exposure and during 19-31 days after exposure to MG.

The control sera were obtained from male university students who were in the same age range as the patients.

The plates of single radial immunodiffusion (SRID) were used for quantitative determination of the serum variables (Behring, Germany). The patients' sera were tested along and concurrently with the sera from the control for measurements of IgG, IgM, and IgA in the SRID plates according to manufacturer protocols and recommendation.

**Statistical Methods –** In this study, the Student T test was employed using SPSS PC and Stat graphics. The P value of < 0.05 was considered significant.

**Table 1. The serum levels of immunoglobulins in patients during one month after exposure to mustard gas .The sera were collected from the patients on day 3, during 4-18 days and during 19-31 days after exposure to this chemical agent.**

Time day(s)	No		Ig	Mean concentration (mg/ml)		SD		P-value
	Patient	Control		Patient	Control	Patient	Control	
3	18	16	IgG	9.5	11.1	2.0	2.2	<0.026
			IgM	1.9	1.5	2.0	1.4	NS
			IgA	2.0	2.2	1.8	0.91	NS
4-18	18	16	IgG	10.6	11.7	2.7	2.5	NS
			IgM	1.7	1.5	1.5	1.1	NS
			IgA	2.4	2.2	1.1	0.91	NS
19-31	18	16	IgG	15.2	11.1	4.2	2.2	<0.001
			IgM	1.4	1.5	0.76	1.4	NS
			IgA	2.6	2.2	0.95	0.91	NS

NS= not significant

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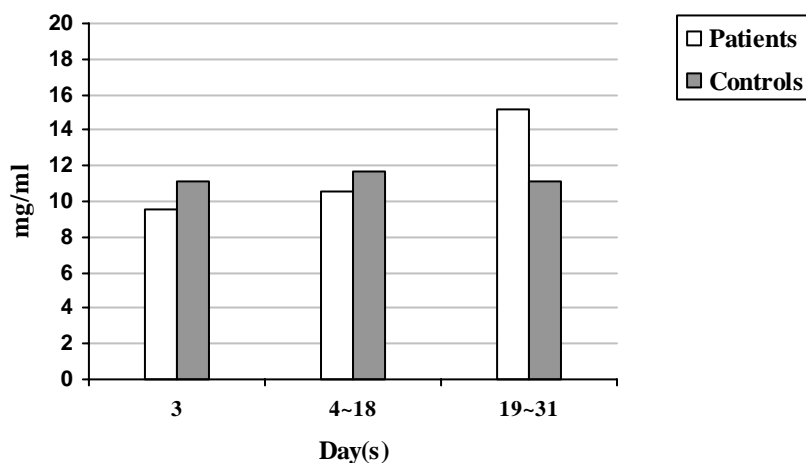


Figure 1. The serum levels of IgG in patients during one month after exposure to mustard gas.

### RESULTS

#### Immunoglobulin G

The measurements of serum IgG by SRID revealed that the levels of this immunoglobulin on day 3 after exposure in patients had decreased significantly comparing to those of the normal controls. ( $P = < 0.026$ ).

The mean serum levels of IgG 4-18 days after exposure was found to increase, however remaining lower than the levels of IgG in the sera of the controls. The sera which were collected from the patients during the 19-31 days after exposure to mustard gas revealed that the levels of serum IgG had increased, surpassing significantly from those of the controls ( $P = < 0.001$ ) (Table 1, Figure 1).

#### Immunoglobulins M and A

The measurements of serum levels of IgM and IgA in patients on day 3, during 4-18 days and during 19-31 days after exposure indicated that the levels of these two immunoglobulins had not changed significantly comparing to those of the normal controls (Table 1).

### DISCUSSION

Among three serum immunoglobulins which were investigated in patients exposed to mustard gas in the battlefield, IgG levels showed noticeable alterations in serum during one month after exposure to this chemical

warfare (Table 1, Figure 1).

The decrease in the serum levels of IgG during the acute phase of exposure to MG in these patients depicts the serum reduction of this immunoglobulin in trauma following thermal injury.<sup>11-13</sup> The decrease in serum levels of IgG in patients on day 3 after exposure can not be attributed to decreased production of this immunoglobulin in these patients since IgG has a long half-life of about 23 days.<sup>14</sup> The reduction of serum IgG in these patients as early as day 3 after exposure to MG can be interpreted in the light of impairments which this chemical agent induce in organs and tissues of exposed victims. Patients exposed to MG in the battlefield developed edema and showed clinical signs of airway obstruction. These were due to extensive exudates which seeped into the respiratory airways<sup>16</sup>. Moreover patients who had been exposed to MG developed blisters of various sizes in different parts of their skins.<sup>15</sup> The observations cited above indicate that following exposure to MG, vasodilatation of capillaries were among the early responses in the organs and tissues that were severely affected by this chemical agent. It is therefore very likely that following vasodilatation and increased vascular permeability, various serum molecules including IgG leaked into certain extravascular parts such as respiratory system and skin blisters.

A study on the immunoglobulin levels in the bronchoalveolar lavage (BAL) fluids of patients who had been exposed to sulfur mustard in the battlefield

showed that only IgG level had increased significantly in the BAL fluid of patients comparing to the IgG level in the BAL fluids of the normal control.<sup>16</sup>

Leakage of immunoglobulins into the skin blisters following thermal injuries<sup>12,13</sup> and in some autoimmune diseases<sup>17,18</sup> have also been shown to occur. Vasodilatation of capillaries usually takes place following release of vasoactive substances from the stimulated cells.<sup>14</sup> It is therefore quite conceivable that vasoactive molecules such as histamine were released in certain organs of the patients as the result of exposure of skin and mucous membranes to mustard gas. However there is no report about the composition of blister fluid in patients who had been exposed to mustard gas neither is any report regarding the kidney status of these patients and whether any immunoglobulin and other proteins had passed through the kidneys into the urine.

The serum levels of IgA in patients during one month after exposure to mustard gas showed alterations similar to those of IgG (Table 1).

However, the alterations of the patients IgA levels comparing to those of the normal controls were not significant. On the basis of available information, it is quite conceivable that IgA as well as IgM did not leak from capillaries into the severely affected tissues and into the skin blisters because of the larger molecular sizes of these immunoglobulins comparing to that of IgG.

The significant increase of IgG in the patients' serum during 19-31 days after exposure to mustard gas is most probably due to (auto) antigenic stimulation of the immune system. It is speculated that extensive tissue injuries and necrosis of cells took place in the organs of patients exposed to mustard gas, resulting to liberation and release of variety of autoantigens. The cells of the immune system were activated; subsequently autoantibodies were formed against these autoantigens. The role of autoantibodies is in fact clearance and elimination of the autoantigens from the internal environment.<sup>19</sup> Stimulation of the immune system by the microbial antigens also could have contributed to antibody production thereby increased the serum IgG in patients. The view that the significant increase of serum IgG in patients was due to antibody production against (auto) antigens is substantiated by the finding that at this particular period of illness in these patients, the complement system also showed

activation with marked increase in CH<sub>50</sub> (unpublished data).

In conclusion, the available data indicate that the humoral immune system of patients as far as production of IgG, IgA and IgM are concerned did not severely impair after exposure to mustard gas. The decrease in serum IgG which occurred few days after exposure to MG was found to be transient and it is highly unlikely to be due to inhibition of antibody production in these patients.

The data in the present study shed light on the certain early events which developed in the acute phase of this trauma. These findings may provide insight about therapeutic measures which should be taken at the early stage of this trauma in order to save the lives of the victims.

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