

## HEMATOLOGICAL FINDINGS OF SULPHUR MUSTARD POISONING IN IRANIAN COMBATANTS

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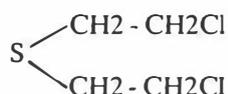
### ABSTRACT

Sulphur mustard (SM) is an alkylating agent that was first used as a chemical warfare agent during the First World War in 1917. SM is readily absorbed from the skin, respiratory and gastrointestinal tract and is distributed to several organs. SM may act rapidly and persistently upon DNA replicating mechanism of the individual cells during mitosis particularly in the hematopoietic system. Of 233 patients with SM poisoning, hematological investigations were performed in 213 of them. Mild changes were observed in red blood cells and its indices. Initial leukocytosis ( $> 11 \times 10^9/L$ ) was observed in 7.2% and leukopenia ( $< 4 \times 10^9/L$ ) in 3.8% of the patients. Marked lymphopenia, neutrocytosis and eosinopenia ( $< 2\%$ ) were found in 36%, 38%, and 25% of the patients, respectively. Bone marrow biopsy in 3 fatal cases revealed marked hypocellularity and dyserythropoietic changes. Apart from the respiratory complications, mortality from SM poisoning is mainly due to bone marrow failure.

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### INTRODUCTION

Sulphur mustard (SM) is an alkylating agent that was first synthesised by Despretz in 1882.<sup>1</sup> The chemical structure is as follows:



SM was first used as a chemical warfare agent on a large scale on July 12th, 1917 by the German army against the French and the Canadian troops near Ypres, Belgium; and since then it has also been called Yprite.<sup>2</sup> Despite strict international prohibition on the production and application of chemical and biological weapons (Geneva Protocol of 1925 and subsequent conventions), the Iraqi army has resorted to the use of the chemical warfare agents repeatedly against Iranian combatants and even Iraqi civilians (Halabjeh) since December 1980.

Thousands of the combatants have become intoxicated by chemical warfare agents and most of them

have been martyred during these attacks during March 1984 in Majnoon Islands, March and April 1985 around Abadan and from February to June 1986 in the southern part of the Arvand river.<sup>3</sup> Almost 2000 war gas intoxicated patients were referred to the university medical centers of Mashad. Of these, 233 cases were studied clinically and paraclinically. Acute toxic effects of SM have been previously reported.<sup>4,5,6</sup> There are only a few reports in the literature on the hematologic effects of SM which may play an important role in morbidity and mortality.

### Brief Toxicology Of Sulphur Mustard

SM is an oily liquid heavier than water. It ranges from colourless to dark brown and is sparingly soluble in water. SM is relatively stable chemically and physically. It gradually hydrolyses in water, requiring 110 minutes for 99% hydrolysis at 20°C and is quickly oxidised to its sulfoxide, a less toxic compound by chlorinated lime. SM makes nucleophilic reactions (slow in water emulsions and fast when dissolved), reacts with N-bases, alcohols, undergoes oxidation,

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Table I- Changes of red blood cell parameters in patients with sulphur mustard poisoning

TEST	NORMAL	NO	BELOW NORMAL	ABOVE NORMAL	L.L.* %	H.L.** %
Hb	12-18 g/dL	212	37	--	17.4	--
Hct	37-54 %	212	12	--	5.6	--
R.B.C	$4-6 \times 10^{12}/L$	212	4	25	1.8	11.8
M.C.V	80-96 fl	212	36	2	16.9	0.9
M.C.H.	26-32 pg	212	22	28	10.3	13.2
M.C.H.C.	32-36 g/dL	212	12	--	5.6	--
E.S.R.	20mm	23	--	--	--	--
Retic.	1-2.8 %	70	10	6	14.2	8.2

\* L.L.: Low level

\*\* H.L.: High level

chlorination, and forms sulphonium salts.<sup>7,8,9</sup>

SM is readily absorbed from skin, respiratory and gastrointestinal tracts and is distributed in several organs. It is rapidly absorbed from the moist parts, such as the armpits and the genital area. Animal experiments show that 90% of the absorbed SM is excreted in the urine as metabolites, 25% to 30% as hydrolysis products, 50% as conjugates with cysteine and 10% as toxic reactive metabolites.<sup>8</sup>

SM produces acute toxic effects only at supralethal

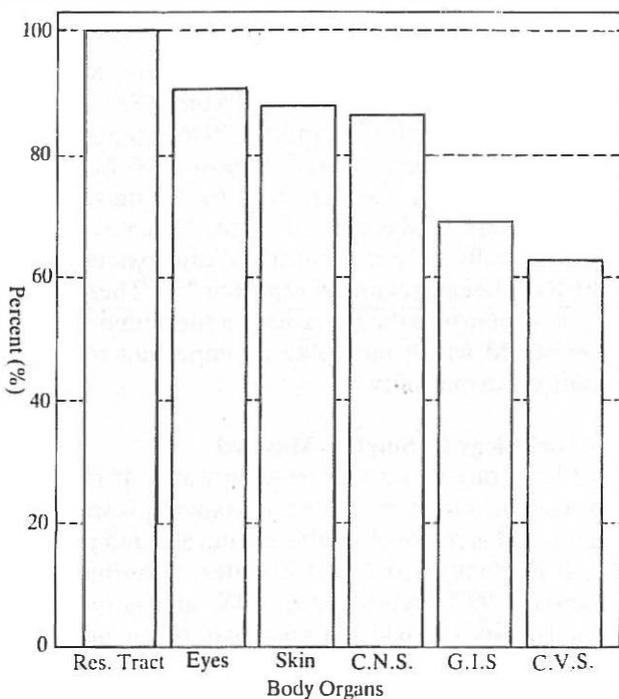


Fig.1: Frequency of toxic effects of Yperite on body organs.

Table II-White blood cells and platelet changes in patients with sulphur mustard poisoning

TEST	NORMAL	NO	BELOW NORMAL	ABOVE NORMAL	L.L.* %	H.L.** %
W.B.C.	$4-11 \times 10^9/l$	207	8	15	3.8	7.2
P.M.N.	40-70 %	201	8	76	3.9	37.8
band	1-2 %	201	--	--	--	--
Lymph	20-45 %	202	72	14	35.6	6.9
Mono	2-8 %	206	10	32	4.8	15.5
Eosin	2-6 %	137	43	22	24.8	12.5
Baso	0-1 %	196	--	3	--	1.5
Platelet	$130-420 \times 10^9/l$	213	2	1	0.9	0.4

\* L.L.: Low level

\*\* H.L.: High level

dosages. Central nervous excitation leads to convulsions and rapid death. The first definite symptoms generally occur in the eyes between half an hour to three hours after exposure. There is acute conjunctivitis, increased nasal secretions, sneezing, sore throat, coughing, and hoarseness. By four to six hours the symptoms become more marked and distressing. There is itching, dusky erythema of the exposed parts and blisters begin to appear in the axillary and genital parts. During the second day, inflammation of the respiratory tract becomes conspicuous in severe cases, leading to bronchopneumonia; and death may ensue between the second and fourth weeks.<sup>1,10</sup>

The initial hematological abnormalities are leukocytosis, neutrocytosis and lymphopenia which may appear during the first few days after exposure. In severe cases WBC gradually falls to leukopenic levels and may even become aplastic. Eosinophil count may be elevated during this period. Biochemical tests are usually not altered significantly.

Late toxic effects including delayed keratitis, mutagenic, carcinogenic, and teratogenic effects of SM have been reported in the medical literature.<sup>1,11</sup>

### MATERIALS AND METHODS

Between February 12th and April 10th 1984, 233 patients with chemical war gas poisoning were referred to the Imam Reza Hospital in Mashad. The patients were Iranian combatants who were gassed in surprise attacks by Iraq and were not able to use the protective clothing or even face masks. The severity of the poisoning varied depending on the distance from the site of explosion and the wind direction. The patients

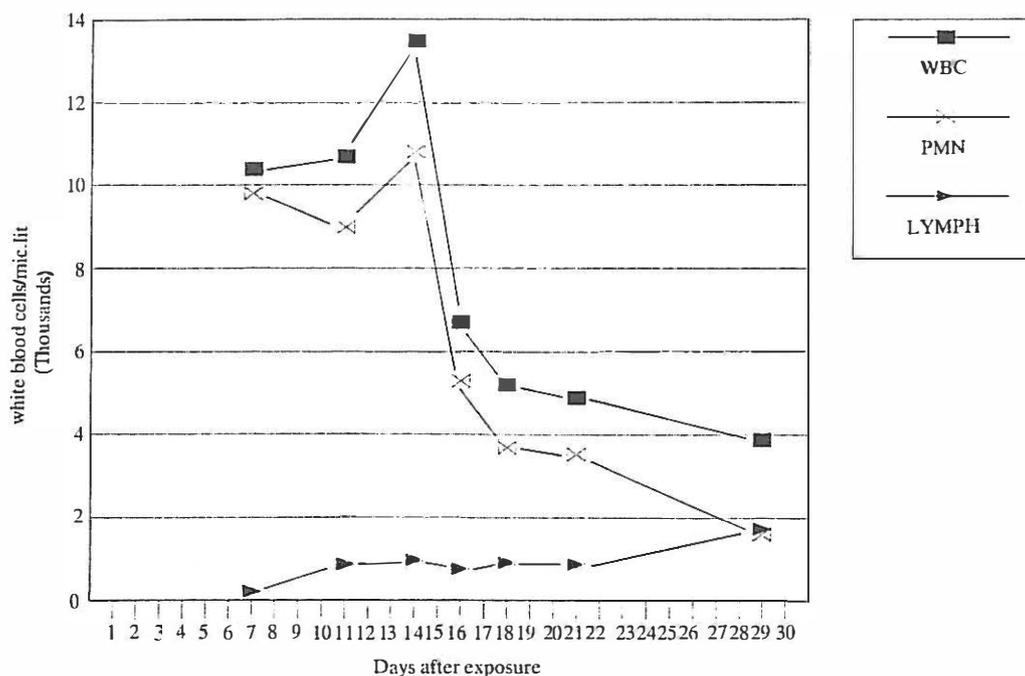


Fig.2: Leukocyte changes of a patient after SM poisoning.

were admitted to the hospital 20 to 48 hours after exposure. The clinical and paraclinical findings have been previously reported.<sup>4,5,6,12</sup> The toxicological analysis of the urine and blister fluid revealed sulphur mustard poisoning.<sup>13</sup> White blood cell counts, red blood cell indices and platelet counts were performed by the Coulter-S, reticulocyte count by supravital staining, and erythrocyte sedimentation rate (ESR) by Westergren method. In one patient bone marrow aspiration was performed before and in three patients biopsy were obtained after death. Cytomorphological study was carried out by routine May-Grunwald Giemsa (MGG), Iron, PAS, reticulon and hematoxylin-eosin stains.

The hematological parameters were obtained in variable numbers of patients ranging from 173 to 213 due to heavy medical load at a given time admission.

## RESULTS

The clinical findings on 233 patients were previously reported.<sup>3</sup> Based on clinical studies the toxic effects on different organs are shown in Fig 1. Of 233 patients only 3 died during the second week after exposure.<sup>5</sup>

The toxic effects of SM on the hematopoietic system are divided into two groups:

- 1- Toxic effects on peripheral blood.
- 2- Toxic effects on bone marrow.

The results of peripheral blood analysis are summarized in Tables I & II.

### Toxic effects on red blood cells

The total RBC count was higher than normal ( $5-6 \times 10^{12}/L$ ) in 25 (11.8%) and lower than normal in 4 (1.8%) patients. The hemoglobin (Hb) and hematocrit (Hct) were low in 37 (17.4%) and 12 (5.6%) patients respectively. The mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were less than normal in (16.9%), (10.3%) and 5.6 percent, respectively as shown in Table I. Hypochromic anemia was seen in 10% and microcytic normochromic anemia in 16.9% hypochromic microcytic anemia was recorded in 5.6% of the patients.

The reticulocyte counts were lower than normal in (14.2%) and higher in 6 (8.5%) patients. Platelet counts were within the normal range except in the 3 fatal patients in which they were low as judged by evaluation of the peripheral smear.

### Toxic effects on leukocytes

The toxic effects of SM were observed on white blood cells (WBC) particularly on lymphocytes and polymorphonuclear leukocytes (PMN) in severely intoxicated patients. There was an initial leukocytosis followed by leukopenia. In severely intoxicated patients, recovery was preceded by improvement of leukopenia and PMN. On the other hand the severe lymphopenia only gradually improved over the next 3 weeks as shown in Fig.2.

In the 3 fatal patients, WBC continued to decline and become undetectable in one of them just prior to

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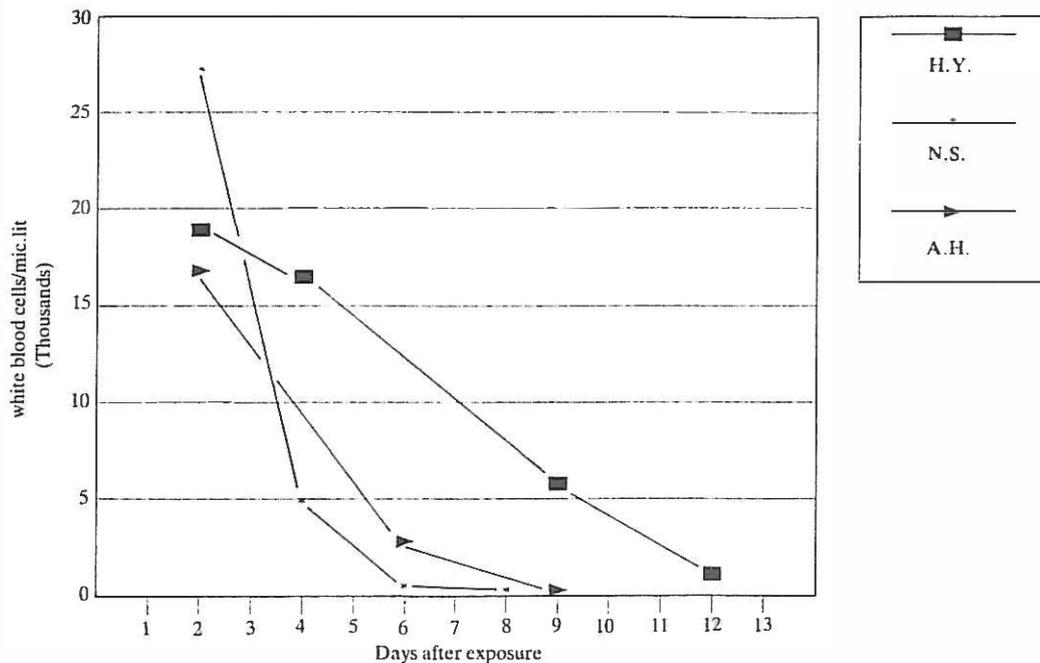


Fig.3. Severe reduction of P.B.L. in three fatal cases of SM poisoning.

death. In the other two, the last WBC counts were reduced to  $0.450$  and  $1.1 \times 10^9/L$  (Fig. 3).

Of 207 patients, 7.2% had leukocytosis and 3.8% leukopenia as shown in Fig. 4. Marked lymphopenia was observed in 72 (36%) patients while during the recovery phase lymphocyte counts increased to  $>40\%$  in 38 (18%) patients (Fig. 5). Initial neutrocytosis was observed in 76 (38%) and following neutropenia in 8 (4%) as shown in Fig. 6. Monocyte and eosinophil count were raised in 32 (15.5%) and 22 (12.5%) patients, respectively. Eosinopenia ( $>2\%$ ) was observed in 43 (25%) patients (Table II).

### Bone marrow

Bone marrow examination showed marked hypocellularity in two of the three fatal cases while in

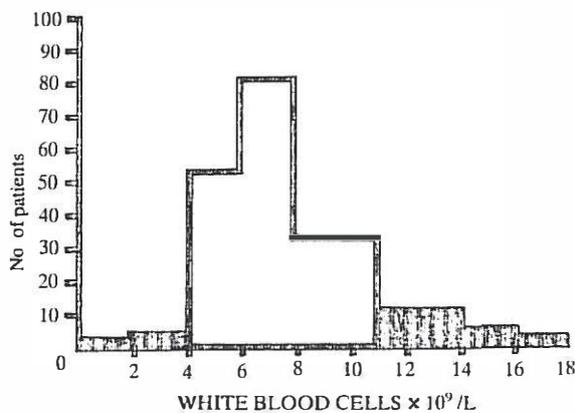


Fig.4. Leucocyte changes in 207 patients with SM poisoning.

one it was normal before and after death. There was also a left shift with only a few mature neutrophils. Megakaryocytes were markedly diminished in the two cases. Dyserythropoiesis (budding, double and multi nucleated and karyorrhexis) was observed in the patient with normocellular marrow (Fig.7).

### DISCUSSION

The toxic effects of SM on the hematopoietic system has not been studied in detail, and only reported as part of the general intoxication.<sup>5,12,14,15</sup> Based on the pre-

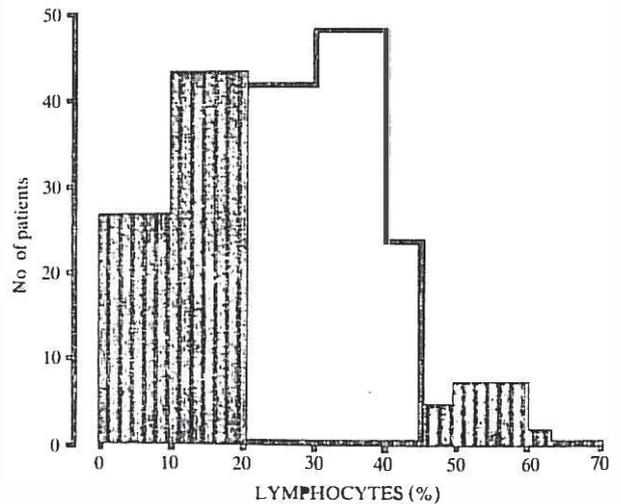


Fig.5. Lymphocyte changes in 207 patients with SM poisoning.

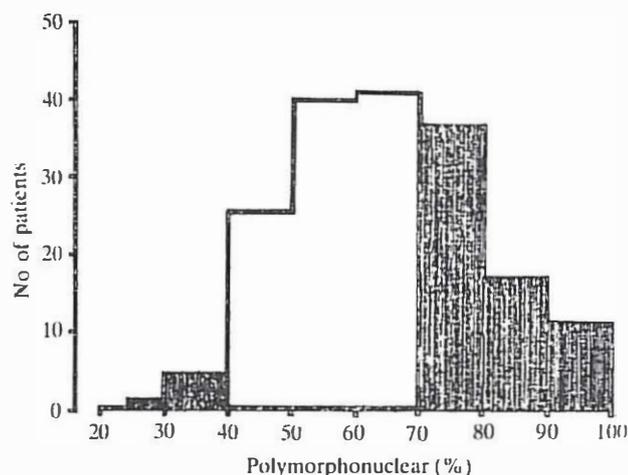


Fig.6. PMN changes in 207 patients with SM poisoning

vious reports and clinical findings, the toxic effects of SM are dose dependent.<sup>5,10,14,15,16</sup> In three fatal cases, only the youngest who had used a protective suit without a face mask, revealed less severe bone marrow suppression as compared with the other two who were not wearing protective devices at all.

The initial leukocytosis is probably a contribution from the marginal pool.<sup>17,18</sup> The lymphocytes are very sensitive to cytotoxic effects of mustards. This may be the reason for depletion of lymphocytes in lymph nodes and the spleen.<sup>19</sup> If the patient survives, lymphopenia may improve. The reason for leukopenia is bone marrow suppression due to direct toxic effects of SM. On the other hand the erythrocytes do not seem to be very sensitive to SM, because of the absence of nuclear material. However, in the severe fatal cases, bone marrow nuclear changes as accelerated pyknosis (20) such as budding, bi- and multinucleated cells and karyorrhexis were observed. Due to the longer life-span of the RBC and less susceptibility to SM, toxic effects on these cells do not occur rapidly in the mild to moderate intoxication. Although the platelet life-span is much shorter (8-12 days compared to 100-120 days for RBC) than the erythrocytes, they did not also appear to be affected in our patients. This may be the reason for notable absence of hemolysis and hemorrhagic diathesis.<sup>21</sup>

SM, a powerful alkylating agent, like its analogue (nitrogen mustard) may act rapidly and persistently upon the deoxyribonucleic acid replicating mechanisms of the individual cell during certain phases of mitosis, such as G cell cycle.<sup>2,22</sup>

Our findings in the youngest martyr revealed that in less severe intoxication, there may be myelodyserythropoiesis (or myelodysplasia) as suggested by the presence of budding, double nuclei and karyorrhexis.<sup>23,24</sup> This patient died because of the adult respiratory distress syndrome (ARDS), pulmonary obstruction and infection, mortality is mainly due

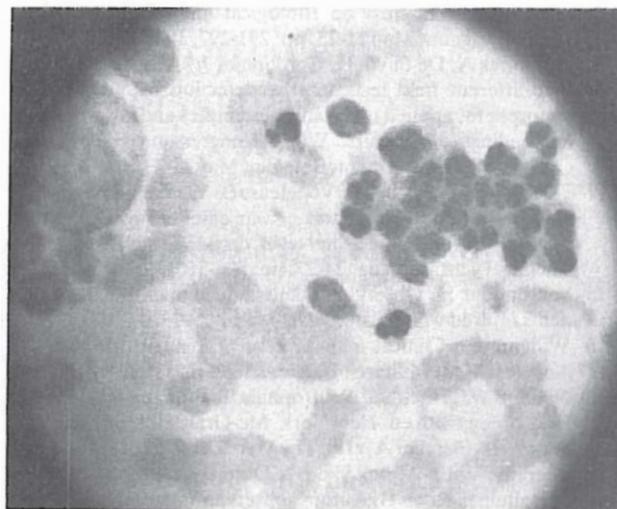


Fig.7. Marked erythroid series changes in bone marrow of a patient with SM poisoning a few minutes before death.

to bone marrow failure. Severe leukopenia and immunosuppression in these patients, predisposes them to septic shock and death.<sup>25</sup>

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