

Original Article

Hematologic and Cytogenetic Investigations of Patients with Severe Chemical Injuries after Exposure to Mustard Gas During Iran-Iraq Conflict

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ABSTRACT

Objective: Sulfur mustard [SM] is a potent alkylating agent with mutagenic properties. It has been widely used in Iran-Iraq conflict. This study assessed the impact of this agent on the hematologic parameters and chromosomal aberration (CA) in the peripheral blood of severely injured Iranian combatants.

Methods: Twenty five patients with severe lung and eye injuries and ten control subjects were included in the study. The subjects of control group were healthy volunteers matched for sex and age. The lymphocytes were cultured by conventional culture methods. Hematologic parameters including CBC, platelets, blood

index and peripheral blood films were studied. Twenty five well-spread metaphases were scored for each sample. Two groups were compared with statistical methods including t-test and Chi Square test.

Results: We found that the mean Hb, Hct, WBC, Platelets, MCV and lymphocytes in the two groups were different ($P < 0.001$). The patient and control groups showed differences in the percent of metaphases containing at least one chromosomal aberration.

Conclusions: This study shows that there is a direct correlation between SM exposure and hematological as well as cytogenetical anomalies.

KEYWORDS: chromosomal aberrations, sulfur mustard

INTRODUCTION

2,2'-Dichlorodiethyl sulfide (sulfur mustard) is a vesicant or blistering chemical agent of historical and current interest^[1]. There is still no effective treatment available to prevent or minimize injury induced by exposure to sulfur mustard (SM)^[2]. This substance has been used in many military conflicts, the most recent being the Iran-Iraq war in the 1980's. The primary effect of SM is the production of serious eye injury and respiratory system damage. Sulfur mustard has been used several times in the last century as a chemical warfare agent^[3] with devastating acute effects such as erythema and blistering on human victims as well as systemic and genotoxic effects^[2]. Up to the present time, however, most of the studies have been focused on SM effects on keratinocytes^[4,5]. Several mechanisms of the toxic effect of sulfur mustard have been proposed^[6]. There is a substantial body of evidence, which suggests the involvement of DNA in the mode of action of sulfur mustard^[7]. Furthermore, sulfur mustard has been shown to possess mutagenic and carcinogenic activity, and also has the ability to produce chromosomal aberrations and a variety of other types of DNA damage^[8]. Blood parameters are very

susceptible to alkylating agents. In addition to severe lymphopenia, destruction of lymph tissues in bone marrow, spleen, lymph nodes and Peyer's plaques are also seen^[9]. SM induces apoptotic/necrotic pattern of endothelial cell death^[10]. Alkylation of DNA by SM alters the function of RNA polymerase and inhibits transcription of mRNA with a consequential effect on translation^[11]. Nitrogen mustard can induce chromosomal aberration (CA) and sister chromatid exchanges (SCEs). Damages induced by nitrogen mustard are non-random. Hot spot in chromosomes of lymphocytes treated by nitrogen mustard is 9q1. An elevated breakage was seen in the chromosome 5,7,11 and 17^[12].

The patients included in this study were Iranian combatants who were injured severely by sulfur mustard gas during Iran-Iraq war between 1985-1988. This study was conducted during 1998-2000, approximately 10 to 12 years after exposure.

Almost all of them suffered from chronic illnesses such as appearance of blisters on some area of their bodies, respiratory system and eye problems (e.g., inflammation). Some of the casualties had problems in their reproduction and digestive system and a few developed malignancies.

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MATERIAL AND METHODS

Patients:

The present study sought to investigate the influence of SM on chromosomal aberration and blood parameters in 25 Iranian combatants who were severely exposed to SM during the Iran-Iraq war. Heparinized peripheral blood samples were obtained from 25 non-smoking subjects with severe sulfur mustard injuries sustained during the Iran-Iraq war and 10 healthy, non-smoking control males. The age of patient and control groups ranged from 29 to 60 years. The subject gave informed consent for venepuncture and completed a detailed questionnaire covering past medical and family history, medication and lifetime occupational history.

Clinical profile:

Almost all of the patients were suffering from chronic illnesses such as appearance of blisters on some area of their bodies, respiratory system such as chronic lung disease and eye problems like conjunctivitis. Some of the casualties had problems in their reproduction and digestive system and a few developed malignancies (e.g. leukemia)

Cytogenetic Methods:

The peripheral blood samples were cultured in RPMI 1640 (Gibco) medium supplemented with 15% fetal calf serum, 1% penicillin-streptomycin, 2% phytohaemagglutinin (Gibco) (complete medium). Two-hour prior to harvest, Colcemid (N-Deacetyl-N-methylcolchicine) was added to culture at a final concentration of 0.1 µg/ml. After harvesting by centrifugation, the cells were subjected to hypotonic swelling in 0.075 M KCL and fixed in 3 changes of methanol/glacial acid [3:1]. The cells were suspended in a small volume of fixative and applied drop-wise onto pre-cleaned slides. The slides were stained with Giemsa for 20 minutes. By selecting 25 well-spread and well-stained metaphases, the results of chromosome aberrations were scored per subject on coded slides by a single observer.

Hematologic methods:

Hematological factors were determined with Sysmex K1000 coulter counter and differential cell counting with x100 lens of optic microscope.

Statistical Methods:

Statistical analysis was performed using one-tailed students t-test and Chi Square^[13]. In this study, SPSS software version 10.0.5 was applied for comparing the patient and control groups. A p-value of <0.05 was regarded as significant. t-student test was used for evaluating differences between the two groups for percentage of cells containing at least one chromosome or chromatid breakage.

RESULTS

Table 1 shows the results of the measurement of blood parameters in those with severe chemical injuries and controls. A statistically significant difference was found between Hb, Hct, MCV, Platelets count, WBC and lymphocyte percent obtained from the patient and control groups (p < 0.0001). In this study, there is a real decrease in the Hb, Hct, MCV, platelets and lymphocytes and an increased in WBC count. There were atypical lymphocytes, band cells, myelocytes, metamyelocytes and prolymphocytes in some of the patients. There were also anomalies in the RBCs of patient group such as anisocytosis, poikilocytosis, target cells, bur cell and ovalocyte. Study of correlation between measured blood parameters and patient's age showed that in all instances, there is no correlation between them. The Chi square test was used for comparing the two groups for cells containing numerical chromosomal aberrations (Polyploidy, aneuploidy, etc). Cells from patients showed high level of hyperdiploidy (40%), hypodiploidy (28%) and hypo-hyperdiploidy (16%). The total cells with chromosomal aberrations are 84% in the patients and 30% in the cells from the control group. The results showed that there are real differences between the patient and control groups, Chi = 23.9 with p < 0.002 (Fig. 1). Percent of cells with aberrations was calculated from dividing

Table 1

Mean comparison of blood factors between patient and control group.

Monocyte	Eosinophyle	Lymphocyte	Nutrophile	Platelets	MCHC	MCH	MCV	WBC	Hct	Hb	Age	-
1.33	2.05	28.24	67.5	128 x 10 ³	34.27	28.40	82.52	7520	41.46	13.84	40.88	Mean in patients
0.88	± 1.02	± 9.74	± 12.8	± 10.5	± 1.30	± 2.16	± 5.22	± 1538	± 6.5	± 2.1	± 8.48	(± SD)
1.60	3.00	35.00	62.2	207 x 10 ³	33.50	26.10	86.50	6180	45	15.01	40.80	Mean in controls
± 0.89	± 2.76	± 8.45	± 8.06	± 30.88	± 0.79	± 8.98	± 5.19	± 1447	± 13.4	± 0.70	± 9.8	(± SD)
- 0.56	- 1.30	- 2.68	1.21	- 2.60	1.69	1.22	- 2.05	2.56	- 2.1	- 2.46	0.02	t-test
NS	NS	S	NS	S	NS	NS	S	S	S	S	NS	t-test result

S for significant; NS for Non-Significant

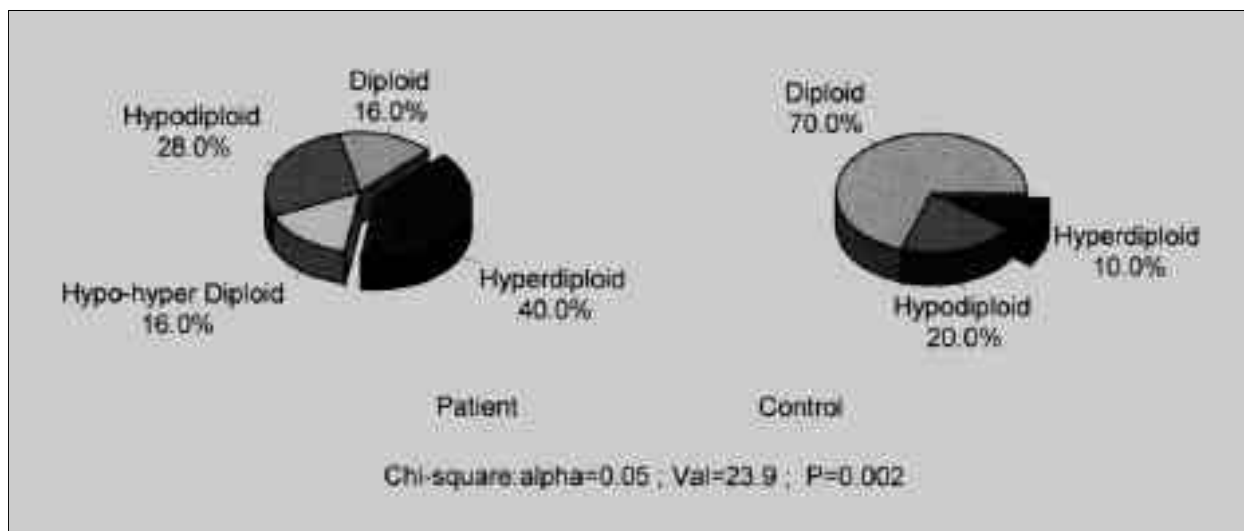


Fig. 1: Numerical aberration (mean comparison)

Table 2

Breakage of chromosomes in injured patients and in controls

Level of confidence	Degree of freedom (D)	t-value	Standard Error (SE)	Standard Deviation (SD)	Mean	No. Variable
99%	95%					
2.76	2.04	28	3.00	2.65	13.2	30.03
				3.78	8.4	11.4
						25
						10
						Breakage % (patient)
						Breakage % (control)

the metaphases containing breakage by the total counted metaphases. The results showed that there is a significant difference between the two groups and the mean breakage percent in those with severe chemical injuries was higher than controls (Table 2).

DISCUSSION

It has been documented that sulfur mustard is a potent mutagen, carcinogen and teratogen^[14]. The toxic effects of mustard include inhibition of mitosis, disturbance in cell cycle phases, nicotinamide adenine dinucleotide (NAD⁺) depletion, and decreased tissue respiration. Most of the toxic effects are related to alkylation of DNA and other critical target molecules^[15]. Depression of cell-mediated immunity (CMI) in Iranian mustard gas-injured patients was observed after one to three years of exposure^[16]. Our research findings suggest the probability of severe damage to bone marrow and weakening of the immune system. Morphological anomalies and immature cells confirm damages to the bone marrow. The re-appearance of the blisters in some of the cases may be due to the release of tissue molecules-linked mustard. The use of alkylated agents has been associated with the subsequent development of acute non-lymphoblastic leukemia, breast, lung and ovarian

cancer, leukemia and cold agglutinin syndrome^[17].

In occupationally exposed workers, there has also been an increase in the incidence of malignancies. There is a report of high incidence of chromosome aberration (CA) and sister chromatid (SCE) exchange rate 11% and 5-18% respectively among the former workers in a mustard gas manufacturing plant. One of them showed an extremely high percentage of missing Y chromosome along with the chromosomal translocation t (9;22) and almost a three fold higher SCE rate compared to control group^[18]. We found that the patients have more chromosomal aberrations that may be due to SM exposure. Chromosome translocations appear to be important events in the development of human malignancies^[19]. It seems the study of CA with blood parameters is a good prognostic indicator and a diagnostic tool to evaluate malignancies. This study tries to find a correlation between the genotoxic effect of sulfur mustard gas and hemato-cytogenetical damage in their body even after 10 to 12 years of exposure.

REFERENCES

1. Smith KJ, Hurst CG, Moeller RB, Skelton HG, Sidell FR. Sulfur mustard: Its continuing threat as a chemical warfare agent, the cutaneous lesions induced, progress in understanding its mechanisms of action, its long term health effect, and new developments for protection and therapy. *J Am Acad Dermatol* 1995; 32:765-776.
2. Borak J, Sidell RF. Agents of chemical warfare: Sulfur mustard. *Ann Emerg Med* 1992; 21:303-308.
3. Robinson JP. The problem of Chemical and Biological warfare, Vol.1: The use of CB weapons. Sweden, Alquist and Wiksell Humanities Press, 1971.
4. Vaughan FL, Zaman S, Scavarelli R, Bernstein IA. Macromolecular metabolism of a differentiated rat keratinocyte culture system following exposure to sulfur mustard. *J Toxicol Environ Health* 1988; 23:507-518.
5. Momeni AZ, Enshaeih S, Meghdadi M, Amindjavaheri M. Skin manifestations of mustard gas: A clinical study 535

- patients exposed to mustard gas. *Arch Dermatol* 1992; 128:775-780.
6. Papirmeister B, Foster JA, Robinson IS, Ford DR. *Medical Defense Against mustard gas*. CRC press. Boca Raton, Florida, USA, 1991
 7. Papirmeister B, Gross CL, Meier HL, Petralli JP, Johnson JB. Molecular basis for mustard-induced vesication. *Fund Appl Toxicol* 1985; 5:134-149.
 8. Fox M, Scott D. The genetic toxicity of nitrogen and sulfur mustard. *Mutat Res* 1980; 75:131-168
 9. Petralli JP, Oglesby SB, Meier HL. Ultrastructural correlates of the protection afforded by niacinamide against sulfur mustard-induced cytotoxicity of human lymphocytes *in vitro*. *Ultrastruct Pathol* 1990; 14:253-262
 10. Dabrowska MI, Becks LL, Lelli JL Jr, Levee MG, Hinshaw DB. Sulfur mustard induces apoptosis and necrosis in endothelial cells. *Toxicol Appl Pharmacol* 1996; 141:568-583
 11. Masta A, Gray PJ, Phillips DR. Effect of sulfur mustard on the initiation and elongation of transcription. *Carcinogenesis* 1996; 17:525-532
 12. Lambert B, Holmberg K, Einhorn N. Persistence of chromosome rearrangements in peripheral lymphocytes from patients treated with melphalan for ovarian carcinoma. *Human Genet* 1984; 67:94-98
 13. Lazutka JR. Replication index in cultured human lymphocytes: methods for statistical analysis and possible role in genetic toxicology. *Environ Mol Mutagen* 1991; 17:188-195.
 14. Pauser G, Aloy A, Carvana M *et al*. Lethal intoxication by wargases on Iranian soldiers. Therapeutic interventions on survivors of mustard gas and mycotoxin immersion. *Arch Belg* 1984; 341-351.
 15. Somani SM. *Chemical warfare agents*. Academic Press Inc. 1992; 14-16.
 16. Zandieh T, Marzban S, Hassiri G, *et al*. Evaluation of Cell-mediated immunity in mustard gas injuries. *Med J Iran* 1990; 4:257-260.
 17. Pedersen-Bjergaard J, Larsen SO. Incidence of acute non-lymphocytic leukemia, preleukemia and acute myeloproliferative syndrome up to 10 years after treatment of Hodgkin's disease. *N Engl J Med* 1982; 307:965-971.
 18. Shakil FA, Kuramoto A, Yamakido M, Nishimoto Y, Kamada N. Cytogenetic abnormalities of hematopoietic tissue in retired workers of Ohkunojima poison gas factory. *Hiroshima J Med Sci* 1993; 42:159-165.
 19. Rowley JD: Biological implications of consistent chromosome rearrangements in leukemia and lymphoma. *Cancer Res* 1984; 44:3159-3168.