

Original Article**Effect of Sulfur Mustard Toxicity on FLT3-ITD Gene Mutation in Sulfur Mustard Veterans**

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ABSTRACT

Background: Sulfur mustard (SM) is a chemical blistering warfare that affects different organs especially hematopoietic system. Prevalence of acute myeloblastic and lymphoblastic leukemia is increased by sulfur mustard exposure. FLT3-ITD mutation can be effective on leukemogenesis. Therefore, the aim of this study was to verify the frequency of FLT3-ITD mutation in the patients who exposed to SM.

Methods: This study was implemented on 42 people poisoned by SM during Iraq-Iran war about three decades ago and is now resident in Mashhad, Iran. The control group included 30 healthy males that are relatives of the patients with first-degree. After DNA extraction, PCR was performed for FLT3-ITD analysis.

Results: By analysis of PCR products, no FLT3-ITD mutation was detected in the patient or control groups. There was no significant difference in hematological factors between the two groups.

Conclusion: Other mechanisms can lead to leukemia in SM exposed persons. Elapsed time after exposure to sulfur mustard can be effective on leukemogenesis, then future more study may be beneficial for early diagnosis of leukemia in SM exposed veterans.

Keywords: FLT3, Iran, Leukemia, Mutation, Sulfur Mustard.

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INTRODUCTION

Sulfur mustard (SM) as a blistering agent is liquid, oily shape, colorless to amber and with neutralization reaction. SM can alkalize of some substances in the human body, such as nucleophile agents (enzymes, membrane proteins in the cytoplasm and nucleus) located in all parts of the cell [1]. It causes paralysis of physiological activities such as in glycolysis, nucleic acids, and protein synthesis, cell membrane function and finally results in cell death [2, 3]. For the first time, SM was used against Belgian troops in the World War I. It was also used in 1980 to 1988 during Iraq-Iran war against Iranian troops by Iraqi forces resulted in more than 100000 toxications [4].

This chemical warfare agent has toxic effects on various organs, especially on the skin, the eyes and digestive, respiratory and hematologic systems. When SM is absorbed in large doses, can lead to damage the hematopoietic cells and for example

cause severe suppression of the immune system and leukopenia. Short-term effects of SM on hematologic system resulted in leukopenia, lymphopenia, neutropenia, and thrombocytopenia [5-8]. Previous studies described some chromosomal abnormalities as a long-term effects of SM toxicity. Long-term effect's study of exposure to SM on workers in SM manufacturing factories showed that the risk of cancer and mortality due to SM exposure was almost five times higher among these workers [9]. SM toxicity increases the risk of acute myeloblastic leukemia (AML) 18-fold and acute lymphoblastic leukemia (ALL) 12-fold. These results are probably due to affinity of SM derivatives to DNA [7].

FLT3 (FMS-Like Tyrosine Kinase III) is a membrane tyrosine kinase receptor that has proto-oncogene role in cells. Mutation of this proto-oncogene is a type of gain of function mutation resulted in activation of the signaling pathway in

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hematopoietic cells and therefore uncontrolled cell growth and proliferation [10]. At the first time, FLT3 mutations identified in AML in 1997. The most common mutation is Internal Tandem Duplication (ITD). Exons of 12 and 11 get involved ITD mutation. [11-13]. The expression of FLT3-ITD mutant in cell lines Cos-7 receptor may cause permanent auto-phosphorylation [14]. Some studies have reported FLT3-ITD mutations in 30%-40% of AML [15, 16], and in 3.2% of ALL [17]. According to increasing risk of malignancies due to SM toxicity, especially hematopoietic cancers and also the role of FLT3 mutation in the leukemogenesis, we evaluated this mutation in Iranian veterans after about three decades of exposure to SM.

MATERIAL & METHODS

This case-control study was performed in Molecular Pathology and Cytogenetic Research Center of Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran in collaboration with Medical Toxicology Research Center and the Janbazan (veterans in person) foundation of Khorasan Razavi Province, Mashhad, Iran, in 2012. The Ethics Committee of the university approved this study.

Veterans' foundation has all evidence and documents about SM toxication including history of disease, extent of exposure and treatment process since exposure to SM. These patients have suffered from the various complications of SM especially in the skin, lungs, and eyes since exposure. Patients with suspicious or very mild history of the exposure were excluded from the study. Finally, 42 patients entered the study. The control group was 30 healthy adult males that were first-degree relatives of the patients and lived in similar circumstance of the patients especially extent of exposure to chemical and toxic agents. Informed consent was obtained from the participants.

Initially, 10 ml peripheral blood was taken from each person in anticoagulant tubes. Sysmex KX-21N (Japan) was used for complete blood count (CBC) and Qiagen kit (Iran) was used for extraction of DNA. Primers were Forward primer: 5'-GCAATTTAGGTATGAAAGCCAGC-3' and Reverse primer: 5'-CTTTCAGCATTGACGG-3'. Primers were synthesized by Metabion Co (Germany). PCR reaction for FLT3 exons 11 and 12 was performed with cycling conditions of an initial denaturation step at 95 °C for 5 min, 35 cycles of 95 °C for 30 sec, 60 °C for 1 min and 72 °C for 90 sec, with a final extension step of 72 °C for 7 min. A wild-type and a mutant sample were also amplified along with the test samples. After the PCR reaction, the reaction products were

electrophoresis on 2% agarose gel and then observed under UV rays. Wild-type samples create a single PCR band at 329bp. Each sample with additional band of bigger size was considered as a mutant one.

Statistical Analysis

Data analysis was done by SPSS software version 16 (Chicago, IL, USA). Independent sample t-test and Mann-Whitney test were used for parametric and non-parametric variables respectively. *P*-value ≤ 0.05 was considered significant.

RESULTS

Age's ranges of patient and control group were 40-60 yr and 20-41 yr respectively. There was no significant difference in white blood cell, red blood cell, hemoglobin, hematocrit and platelet count between patient and control group (Table 1). Electrophoresis of PCR products that incubated with restriction enzyme, showed that both of the patient and control group's products were in the range of 324 bp (Fig. 1). Then there was not FLT3-ITD mutation in the groups.

Table 1. Hematologic values in 42 patients with long-term complications of sulfur mustard toxicity and 30 normal controls.

Parameter	Patient group	Control group	<i>P</i> -value
	Mean \pm SD (Range)	Mean \pm SD (Range)	
WBC ($10^9/L$)	6.61 \pm 1.42 (4.2-10)	6.29 \pm 1.30 (3.8-8.8)	0.338
RBC ($\times 10^{12}/L$)	5.62 \pm 0.87 (4.61-9.96)	5.70 \pm 0.50 (4.87-7.24)	0.650
Hemoglobin (gr/dL)	15.9 \pm 1.58 (12.7-21)	16.2 \pm 0.95 (14.6-18.2)	0.382
Hematocrit (%)	46.0 \pm 4.10 (38.3-59.7)	46.5 \pm 2.99 (41.3-53.5)	0.583
Platelet ($\times 10^9/L$)	237 \pm 49.68 (166-388)	241 \pm 50.54 (161-348)	0.737

WBC: White blood cell, RBC: Red blood cell

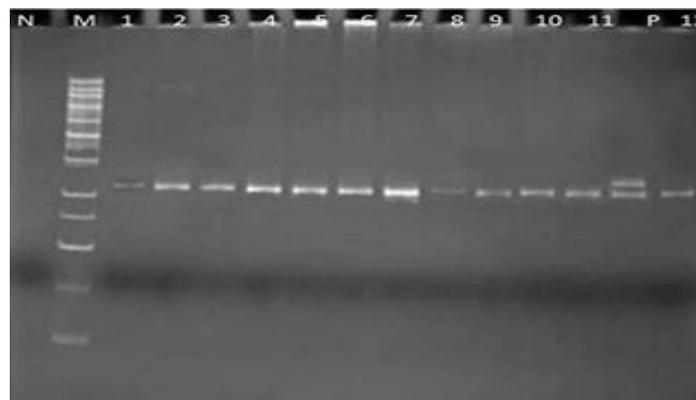


Figure 1. Electrophoresis of PCR products in the control (line 1-6) and patient (line 7-12) groups. Any of patient or control groups did not show FLT3-ITD mutation. M= marker, P= positive control with additional band of bigger size, N= blank or without sample.

DISCUSSION

SM is a toxic chemical warfare creating inhibition of mitosis, disturbance in cell cycle and reduction of tissue respiration and therefore leads to mutation, cancer and fetal malformations [18-21]. A malignancy creates by the clonal proliferation of a precursor cell secondary to DNA damages with different agents such as SM. Normal hematopoiesis is controlled by some factors that do this by binding to tyrosine kinase receptors like FLT3 receptor. One of the frequent mutations in leukemia is FLT-3 mutation that causes autonomous proliferation of hematopoietic precursor cells. Importance of these mutations is the role of FLT-3 in proliferation of the precursor and therefore in leukemias pathogenesis [22-25]. Mutations, monosomy 7 and trisomy 8 that are chromosomal abnormalities are important in leukemia pathogenesis [26] and in addition to, aneuploidy was observed in lymphocytes of patients with SM toxication [27]. Thus increased risk of tumorigenesis may be due to these changes.

Toxication with SM could have some DNA damages and therefore increase risk of tumorigenesis, we did not observe any FLT3-ITD gene mutation in the patients exposed to SM during the Iraq-Iran war around 25-30 yr ago. Accumulation of different many mutations effect on tumorigenesis, gradually [28]. Lately occurring with FLT3-ITD mutation or other genetic damages may be reason for lack of FLT3-ITD mutation in our study.

Changes of genes such as cell growth, proliferation and differentiation have been shown in leukemias. As a short time effect after exposure of SM, GM-CSF and IL-6 expression increase [29]. This interleukin could inhibit programmed cell death in some tumor cells resulting increased risk of tumor cell proliferation [30]. Indeed, high level of the GM-CSF receptors expression has been shown in leukemic cells [31]. These effects of SM on these growth factors, could lead to alteration in myeloid precursors in bone marrow and provoke them to neoplastic proliferation. SM could decrease the level of some anti-inflammatory cytokines such as transforming growth factor β 1 and β 2, On the other hand, increasing number of myeloid cells in AML is caused by decrease of this cytokines [32]. This information shows that SM may increase the risk of leukemia by various mechanisms.

Other mechanisms of tumorigenesis in patients with SM toxication are necessary to survey. Lack of FLT3-ITD mutation in the patients is inconsistent with another study that reported no FLT3-TKD835 (tyrosine kinase domain 835) mutation in SM-exposed veterans [1]. We suggest to future more

study on the veteran about leukemia causer mutation.

CONCLUSION

Although increased risk of some neoplasms, especially hematologic malignancy increased by SM, we did not observe FLT3-ITD Mutation in the patients. Presumably, other mechanisms of leukemogenesis result in increased risk of leukemia intoxication of SM. Because of importance of health of SM toxicity veterans, analysis of other genetic alterations that cooperate in leukemogenesis be done in future study.

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