Original Article

Evaluation of JAK2 (V617F) Mutation in Iranian Veterans with Delayed Complications of Sulphur Mustard Poisoning

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ABSTRACT

Background: Sulfur mustard was the most widely applied chemical warfare agent by the Iraqi army in Iran–Iraq war (1983-1988). Considering the role of sulfur mustard toxicity in hematopoietic neoplasms and also new role of JAK2 mutation in these neoplasms, we assessed this mutation and delayed hematologic complications in veterans exposed to sulfur mustard.

Methods: This case control study was performed in Mashhad University of Medical Sciences, Mashhad, Iran in collaboration with Janbasan Foundation of Khorasan Razavi, Iran in 2012. The case group consists of 42 patients who exposed to sulfur mustard about 30 yr ago and the control group includes 30 healthy persons. For all subjects complete blood counts and ARMSpolymerase chain reaction for JAK2 (V617F) mutation was carried out. Data were analyzed by statistical software using independent sample t-testand Mann- Whitney U test.

Results: JAK2 (V617F) mutation was detected, neither in the sulfur mustardveterans nor in the control group. Moreover no significant difference was detected in hematologic parameters between the two groups.

Conclusion: Despite sulfur mustard can increase risk of tumor genesis especially hematologic neoplasms but this is probablyas result of other genetic mechanism apart from JAK2 mutation. Considering the health and importance of preventive measure for the sulfur mustard victims, we suggest other genetic aspects of tumor genesis to be assessed in these patients.

Keywords: Hematologic, Iran, JAK2, Mutation, Sulfur Mustard.

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INTRODUCTION

Sulfur mustard (SM) as the most widely used chemical warfare agent by the Iraqi army in Iran–Iraq conflict, resulted over 100,000 chemical casualties during 1983-1988 [1-4]. SM is an alkylating agent by the many short and long toxic effects on various organs especially the skin, eyes, hematologic, respiratory and gastrointestinal systems [1, 5-7]. Early hematologic complications on SM toxicity during the war were as leukopenia, lymphopenia, neutropenia and the bone marrow hypoplasia [1, 5].Previous reports [1, 5-7] show that SM exposure can cause some chromosomal abnormalities such as chromosomal breakage and, the hypo- and hyperploidy. These Reports also show that the risk of acute myeloblastic leukemia (AML) in the SM toxicity is 18 fold and acute lymphoblastic leukemia (ALL) is 12 fold comparing to a normal population. This is probably because of the particular affinity of SM derivatives for DNA molecules [5]. Although genetic damages such as mutations are at the heart of carcinogenesis however, they may be acquired by the action of environmental agents, such as chemical warfare.

Janus Kinase 2 (JAK2) mutation has been considered remarkable in hematologic neoplasms especially in myeloprolifrative neoplasms in recent years [8, 9].

Some of the Iranian veterans still suffer from SM complications and it may be used again in the future war or terrorism. Considering the role

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of SM toxicity in tumor genesis especially hematopoietic neoplasms and also the new role of JAK2 mutation in these neoplasms, in this study, we evaluated the mutation and delayed hematologic complications in this rare group of patients after about three decades of exposure to SM.

MATERIALS AND METHODS

This case control study was performed in collaboration with Janbasan Foundation of Khorasan Razavi, Iran in Cancer Molecular Pathology Research Center of Ghaem Hospital (a big teaching hospital located in Mashhad, Khorasan Razavi Province, Northeast Iran) in 2012. Janbasan Foundation of Khorasan Razavihas recorded all documents about toxicity of patients with chemical agents, the medical history, physical examination and all diagnostic, therapeutic and complications of victims after exposure to SM up to now. Cases with suspected history or very mild exposure with SM were omitted from the study. Forty two out of 72 male patients recorded in this center with more than 40% disability due to SM complications, included in this study (the SM toxicity group). These patients were suffering from various complications of SM especially the lungs, the skin and eyes since exposure (1983-1988). The control group was 30 healthy males chosen from the first degree of the patients matched for tobacco smoking.

After approving by the local Ethical Committee, obtaining written informed consent and a short medical history from the patients and control groups, 10 ml blood was taken in EDTA-K2 tubes.

For all subjects, complete blood counts (CBC) were done and with preparing peripheral blood smears, differentiated cell counts were determined. Then, DNA was extracted according to standard methods and ARMS PCR analysis for JAK2 (V617F) mutation was carried out by ABI Veriti PCR Machine (Applied Biosystems, USA).

Statistical Analysis

Data were analyzed by SPSS, Version 11.5 (Chicago, IL, USA). Initially, descriptive analysis and then comparative analysis of variables were performed. Means of the continuous variables between the two groups were compared using independent sample *t*-test or its non-parametric equivalent, Mann- Whitney U test. A *P*-value $\leq$ 0.05 was considered to be significant.

RESULTS

The patients' age range was 40-60 yr and in the control group was 20- 41 yr. We did not detect any JAK2 (V617F) mutation in the SM toxicity or the control group. Table 1 shows hematologic values in SM and control group. As shown, these values did not have a significant difference between two groups.

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

Parameter	Patient group Mean ± SD (Range)	Control group Mean ± SD (Range)	*P-value*
WBC ($\times 10^9$/L)	6.61 ± 1.42 (4.2-10)	6.29 ± 1.30 (3.8-8.8)	0.338
Neutrophil ($\times 10^9$/L)	3.79 ± .96 (2.14-6.30)	3.44 ± 0.95 (1.98-5.02)	0.134
Lymphocyte ($\times 10^9$/L)	2.3198 ± .69 (1.32-3.96)	2.3527 ± 0.59 (1.30-3.64)	0.834
Monocyte ($\times 10^9$/L)	0.40 ± 0.19 (0-0.91)	0.3961 ± 0.17 (0.18-0.97)	0.775
Eosinophil ($\times 10^9$/L)	0.08 ± .11 (0-0.63)	0.08 ± 0.09 (0-0.39)	0.969
Basophil ($\times 10^9$/L)	0.004 ± .01 (0-0.09)	0.004 ± .01 (0-0.07)	0.760
RBC ($\times 10^{12}$/L)	5.62 ± 0.87 (4.61-9.96)	5.70 ± 0.50 (4.87-7.24)	0.650
Hemoglobin (gr/dL)	15.9 ± 1.58 (12.7-21)	16.2 ± 0.95 (14.6-18.2)	0.382
Hematocrit (%)	46.0 ± 4.10 (38.3-59.7)	46.5 ± 2.99 (41.3-53.5)	0.583
Platelet ($\times 10^9$/L)	237 ± 49.68 (166-388)	241 ± 50.54 (161-348)	0.737

DISCUSSION

Tumor genesis is a multistep process and genetic damages lies at the heart of it. These genetic damages (or mutations) can be acquired by the toxic and chemical agents such as SM [8]. The SM toxicity is done partly by the nucleic acids and proteins alkylation. Despite it, the exact mechanisms are not clear. One important reason of this toxicity is the reaction of SM with DNA, forming both DNA monoadducts and inter-strand cross links. These damages can lead to cell death by apoptosis [10-12]. Indeed, cells react to genotoxic damage by activation DNA damage signaling cascades and using specific DNA repair pathways for repairing it. Overlay, this compound can lead to apoptosis, mutations and impairment of natural repair mechanisms of DNA [10, 13-15] and increase risk of malignancy such as lung cancer [5, 16].

JAK2 is a cytoplasmic enzyme with tyrosine kinase activity and JAK2-signal transducers and activators of transcription (STAT) pathway has an important role in signal transmission for cytokines and growth hormones. The mutation causes phenylalanine amino acid to be substituted for valine at position 617 of JAK2 (V617F). The mutation activates transcription factors which promote the growth factor–independent proliferation and survival of the hematopoietic precursors [8, 9]. JAK2 mutation and aberrant activation of the JAK/STAT pathway play an important role in hematologic neoplasms especially in high proportion of myeloprolifrative neoplasms such as polycythemia vera and myelofibrosis [17, 18]. JAK2 mutation can be seen with less frequency in lymphoma and acute lymphoblastic leukemia [17, 19]. Meier et al. detect this mutation in 35% primary mediastinal B-cell lymphomas, 33% Hodgkin's lymphomas,19% angioimmunoblastic T-cell lymphomas [17].

Ghanei et al., evaluated 665 war victims (mean age 34 ± 3 yr) after 12 yr exposed to SM during Iraq–Iran war. They observed two cases of chronicmyelocytic leukemia (CML) in 2 consecutive years and concluded that the incidence of CML among war veterans was significantly greater than in the general population. In this study also increased frequency of other chromosomal abnormalities such as pseudodiploidy and hyperdiploidyin these patents were seen [16]. Hassan et al. also detected high frequency of chromosomal abnormalities such as hyper or hypoploidy in patients with severe toxicitywith SM (but not in the mild toxicity) during Iraq–Iran war [5].

Despite this role of SM carcinogenesis and especially role of SM and JAK2 mutation in hematologic neoplasms like CML, ALL and AML, we did not detect any JAK2 mutation in these patients suffering from SM complications after about three decades of SM exposure. This may be for this reason that increased risk of hematologic neoplasms in these patients are not because of JAK2 mutation or this mutation may be occurred in the last period of multistep pathway of a tumorgenesis.

Hematologic complications of SM divided to short and long term consequences. During a few days to months of exposure, we have leukopenia, neutropenia, lymphopenia, thrombocytopenia and pancytopenia; however, years after exposure most of patients have stable state and theirs CBC and blood smears have not any difference with normal population [5]. Moreover hematologic values were like the control group, after about three decades of exposure to SM (Table 1). Severe polycythemia (Hemoglobin 21 gr/dl and RBC 9.96×10^{12}/L) was observed in one patient and the reason probably was due to respiratory complications of SM and subsequent hypoxemia resulted increased rennin production and erythrocythosis (secondary polycythemia).

In a study of hematologic values on 665 SM victims a minor difference (without clinically important) in these parameters was found between the patient and control groups [16]. WBC was similar in the two groups; however, hemoglobin was a little lower and platelet a little higher in the control group. The other study, for determination of delay hematologic complications of SM on Iranian veteran, revealed that WBC, RBC, hemoglobin and platelet counts were higher in SM toxicity group compared with the control group [1].

This minor difference in the results of various studies can be due to different severity of SM complications and different time of studies since SM exposure, probably not because of direct effect of SM on bone marrow hematopoiesis.

CONCLUSION

Although SM can increase the risk of tumorgenesis especially hematologic neoplasms but this may be due to other genetic mechanism

than JAK2 mutation. Considering the health and importance of preventive measure for the sulfur mustard victims, we suggest other genetic aspects of tumor genesis to be assessed in these patients.

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