IJBC 2017; 9(4): 121-124

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

ORIGINAL ARTICLE

Prostate Specific Antigen Level in Exposure to Sulfur Mustard

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ARTICLE INFO

Article History:
Received: 26.04.2017
Accepted: 03.09.2017

Keywords:
Prostate specific antigen
Sulfur mustard
Oxidant agents
N- acetylcysteine
Prostate cancer
Antioxidants

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ABSTRACT

Background: Oxidants and inflammation agents are predisposing factors for the development of prostate cancer. As a chemical warfare, sulfur mustard (SM) can cause cancer through various pathways mainly increased production of oxidants and inflammation. Due to high incidence of cancer in SM victims, concentration of prostate specific antigen (PSA) in SM victims was evaluated and compared with the control group.
Methods: This study was conducted on 150 subjects exposed to SM as the Iranian chemical victim group and 150 non-exposed healthy subjects matched for age and sex, as the control group. The serum concentration of PSA was measured by Enzyme-linked Immunosorbent assay.
Results: According to spirometry results, the SM exposure rate in the chemical victim group was mild, moderate, and severe in 66%, 27% and 7%, respectively. The mean time elapsed from exposure to SM in case group was 30 years. 100% of the victims had consumed N-acetylcysteine for alleviating symptoms due to exposure to SM. Decreased concentration of PSA in SM victims compared with control group was observed; even non-significant. (0.728 vs 0.844 ng/ml, P=0.103).
Conclusion: PSA concentrations were expected to increase in SM victims, but in our study an opposite result was observed. It is assumed that consumption of N-acetylcysteine with known anti-inflammatory features, mild exposure and shorter period of time elapsed from exposure to SM may be the reasons for this results. Further studies on these subjects seem to be necessary to prove the efficacy of antioxidants such as N-acetylcysteine in prevention of prostate cancer in subjects who have the history of SM exposure.

Please cite this article as: Panahi Y, Alikhani MY, Rafiee M, Saadat AR, Rafieemehr H, Abbasi M. Prostate Specific Antigen Level in Exposure to Sulfur Mustard. IJBC 2017; 9(4): 121-124.

Introduction

Sulfur mustard (SM) gas has been used in past decades as a chemical warfare weapon, although the use of such weapons has been prohibited by international organizations.[1] The most prominent case of mustard gas use was in the Iran-Iraq imposed war between 1980 and 1988 and there are still a large number of chemical victims who suffer from complications of this gas.[1] Skin, eyes and lungs are the initial organs of gas exposure which are also the major damage sites followed by the gastrointestinal tract and hematopoietic system.[2,3]

SM is a strong alkylating agent known as a mutagenic and carcinogenic agent.[4] It attacks the neucleophilic sites in the cell, which accounts for mutagenic and carcinogenic effects of SM.[5] Oxidants are normally produced in the cells and can damage nucleic acids, proteins, and lipids if not neutralized.[6] Oxidants are carcinogenic factors accused of developing and progression of several cancers

and even metastatic potential of cancers such as prostate cancer.[7] Sulfur mustard is capable of increasing the production of superoxide anions (as oxidant agents) in the cells.[8] In high concentrations, SM can severely reduce the activity of catalase and glutathione peroxidase (antioxidant agents) in cells. Inhibition of the antioxidant system by SM increases the concentration of hydrogen peroxide (H2O2), superoxide anion, and lipid peroxides in cells leading to cellular damage.[6] SM can decrease serum levels of TGF-β1 and TGF-β2, which are anti-inflammatory cytokines with anti-tumor activity.[9] Inflammation in the body affects the progression of prostate cancer from carcinogenicity up to resistance against cytotoxic treatments. Exposure to SM causes increased production of interleukin-6 (IL-6).[9] IL-6 and CRP (as inflammatory agents) are recognized as markers of resistance to treatment of prostate cancer.[10] PSA is a natural serine protease and the most useful clinical marker associated with prostate cancer. Early diagnosis and even prevention of prostate cancer is a valuable strategy to save the life of patients and reduce their treatment expenses. Food and Drug Administration (FDA) recommends a combination of digital rectal examination and measurement of PSA tumor marker in men over age 50 with no family history of prostate cancer and those over 40 with a family history of prostate cancer. PSA is synthesized in small amounts in a healthy normal prostate, in moderate levels in inflamed hyperplastic prostate and in high amounts in a malignant prostate.[11]

Due to the increased production of oxidants and inflammation due to SM in chemical victims, we aimed to assess PSA levels in patients exposed to SM and compare with control group.

Materials and Methods

This cross-sectional study was conducted in 2015-2016 on 150 subjects exposed to SM as the Iranian chemical victim group and 150 non-exposed healthy subjects matched for age and sex, as the control group. The injury of chemical victims was confirmed by Iranian Janbazan Foundation. This study was approved by the Medical Ethics Committee of Hamadan University of Medical Sciences, Hamadan province, west of Iran. Patients with malignancy, those undergoing chemotherapy or radiotherapy, smokers, and patients with benign prostatic disease were excluded from the study.

Five mL peripheral blood was taken and centrifuged at 2000 rpm for 20 min at 4°C to isolate the serum. The samples were aliquot, labeled and stored in -80°C freezer until the experiments. ELISA (Enzyme-linked Immunosorbent assay-Monobind Co, USA) kit was used to measure the serum level of PSA based on instructions of the manufacturer. ELISA reader (Rayto Microplate reader, Germany) was used to determine the concentration of PSA.

Drugs that were used by the case group included: N-acethylcysteine with a palliative effect on symptoms of SM intoxications,[12] Fluticasone as inhaled corticosteroid,[13] Salmeterol, a drug for asthma with β2-adrenoceptor agonist activity for alleviating symptoms of chronic obstructive pulmonary disease [14] and Azithromycin, an antibiotic for respiratory tract infections.[15]

The data from the control group (unexposed) and exposed group were compared using t-test. Correlation between the levels of tumor markers with age was calculated on the basis of Pearson correlation coefficient. Statistical analyzes were performed using SPSS software, version 13.

Results

The mean age of the control and chemical victim group was 47.74±5.45 and 49.12±3.83 years, respectively. None of the subjects in the two groups were smokers, had a history of chemotherapy, radiotherapy or prostate disease. According to the results of the spirometry test, the SM exposure rate in the chemical victim group was mild, moderate, and severe in 66%, 27% and 7% of cases, respectively. The mean time elapsed from exposure to SM in chemical victim group was 30 years. All of patients (100%) in chemical victim group had consumed N-acetylcysteine and some of patients had consumed some other drugs associated with SM symptoms, including fluticasone and salmeterol sprays, as well as azithromycin. But none of controls had consumed these drugs, especially N-acetylcysteine

The PSA serum levels showed an insignificant decrease in the chemical victim group compared to control group (0.728 ± 0.669 vs 0.844 ± 0.497 ng/ml; p-value: 0.103) (Table 1). In the control group, there was a significant correlation between age and PSA serum levels so that increasing age caused increasing PSA levels (p-value: 0.00, r: 0.631) (Figure 1).

Discussion

International Agency for Research on Cancer (IARC) has claimed SM as a carcinogenic agent and a known risk factor for lung cancer in the occupations associated with exposure to SM.[16] Based on genetic studies, exposure

Table 1: Descriptive data on age and PSA concentration in case and control group

	Exposed		Unexposed	
	PSA (ng/ml)*	Age (year)	PSA (ng/ml)	Age (year)
Mean	0.728	49.12	0.844	47.74
SD	0.669	3.83	0.497	5.45
Variance	0.44	14.72	0.247	29.79
Min	0.00	40.0	0.15	38.0
Max	3.16	60.0	3.13	61.0

*PSA difference between groups, P value was 0.103. ANOVA test was used to compare the mean of parameters between the experimental groups.

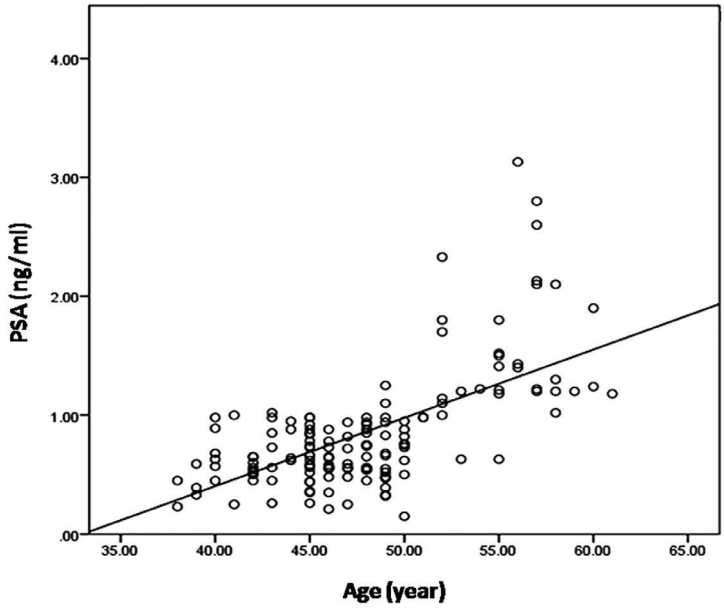

Figure 1: Positive correlation between age and PSA concentration in the control group (Pearson correlation, r: +0.631, P<0.001)

to SM causes increased risk of chromosomal breakage and hyperdiploidy.[9] Aneuploidy of peripheral blood lymphocytes in patients suffering from SM poisoning can be indicative of increased risk of tumorigenesis in such patients.[9] According to one study on 1267 chemical victims of World War in Great Britain who were exposed to sulfur mustard, it was found that the number of lung cancer deaths in these victims was nearly twice more than the control group.[17] In a study of 3530 workers of SM factory in UK between 1940 and 1961 (which continued until 1985 to investigate the mortality rate), a significant increase was found in mortality due to cancer of gums and mouth, pharynx, esophagus, stomach, larynx, and lung compared to national statistics and mortality risk was directly proportional to the rate of exposure.[3] Oxidative stress is considered as an important factor in the development and progression of prostate cancer.[18] Selenium-containing supplements (required by the antioxidant system) reduce the incidence of prostate cancer. Several studies have noted the impact of lipid peroxidation in the development of prostate cancer.[18] Due to the effect of SM (i.e. increased production of oxidants and inflammatory cytokines) on the development of cancers, PSA concentration was likely to be increased in those exposed to SM compared with the control group. In addition, a direct relationship was found between aging with increase in PSA concentration, which seems to be quite logical; however, this relationship was not observed in chemical victims. The possible reason for this result could be the exposure rate of victims which was classified according to the spirometry results. The majority (66%) of those exposed to sulfur mustard suffered from mild complications (27% moderate and 7% severe). Perhaps a higher rate of exposure would lead to different results.

N-acetylcysteine (NAC) is an antioxidant drug inhibiting the oxidative damage to DNA and lipid peroxidation via reduction of free radicals.[19] This drug was consumed by chemical victims of our study as a mucolytic; alleviating the inflammation in respiratory tract. NAC can prevent the proliferation of malignant cells by inhibiting or activating certain signaling pathways in prostate cells.[19] Consumption of NAC could be suggested as a protective factor in these patients to reduce PSA levels.

Vitamin D is an antioxidant and a regulator of the immune system which inhibits severe inflammatory reactions. In people exposed to SM, serum vitamin D levels and the number of lymphocytes was reported to be reduced.[20] Inflammatory reactions and reduced vitamin D levels affect the pathogenesis of prostate cancer and could possess contributions in its progression. Epidemiological studies indicate reduced levels of vitamin D in patients with prostate cancer.[21] IL-8 is an important cytokine of the immune system which is involved in disorders such as acute and chronic inflammation, lung disease and cancer.[22] The levels of this cytokine which is effective in the prevention of cancer is shown to be reduced in the victims exposed to SM.[22]

In this study, none of the patients suffered from cancer and PSA levels were all within normal ranges. Studies on increased incidence of cancer in subjects exposed to SM have examined these patients several years after exposure. For example, the study of Nishimoto was conducted on SM factory workers and reported a nearly five-fold risk of developing cancer after 35 to 50 years of exposure,[16] while our study was performed approximately after 30 years. Therefore, time appears to be a determinant factor and it is recommended to conduct studies with respect to cancer in future on these subjects.

Conclusion

SM can exert its carcinogenic effects in different ways including effect on DNA, inflammation, and oxidant agents, which is indicated by several studies in those exposed to SM. However, according to the long-term

effects of SM, more accurate studies in future on such subjects are highly recommended.

Acknowledgments

The authors would like to acknowledge Hamadan and Baqyiatallah University of Medical Sciences for providing financial support for this study.

Conflict of Interest: None declared.

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