

# Serum Free Testosterone Levels Among Sulfur Mustard Induced Asthma In Iranian War Veterans 15 Years Postwar: A Case Control Study

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**D**ata available implies that certain systemic chronic inflammatory disorders and a few pulmonary diseases affect testosterone (T) biosynthesis. The status of free testosterone (FT) has not yet been determined in sulfur mustard (SM) induced chronic persistent asthma in Iranian veterans for the long term effects. The aim of the study was to assess the status of FT levels on SM induced asthma patients and to compare it with that of non-exposed asthmatics and healthy subjects; our goal was also to determine the frequency of hypogonad hypogonadism in the target population.

**Materials and Methods:** Protocol of study was based on random selection of target population using a self-report questionnaire, physician diagnosed asthma, and ratification of SM exposure. Two control groups, age and sex-matched, were enrolled accordingly as the non-exposed asthma and healthy subjects respectively. Serum samples of FT, Follicle Stimulating Hormone (FSH), and Luteinizing Hormone (LH) were measured and documented.

**Results:** Forty-three male, war veterans, exposed to chemical warfare were enrolled as the case group, mean age 53.95±6.80 years. The mean serum FT levels were 15.70±10.54 pg/mL, the levels of 32.6% of subjects being below the lower normal range. The mean serum values of FSH, LH,

and were 11.91± 9.21 mIU/mL, and 10.33±7.46 mIU/mL, respectively. There were 64 non-chemical asthmatics in the patient group, mean age 52.67±6.44 years. Mean FT levels were 16.97±10.15 pg/mL; 22.2% of the asthma control group had low FT levels. The healthy control group had 46 subjects. The mean FT levels were 22.73±8.30pg/mL. The ANOVA and post hoc (Tukey HSD) tests were used to compare the means of groups. Significant differences were observed between the case and non-exposed asthma groups and the healthy control group.

**Conclusion:** The high significance of low FT was notable in the case and asthma control groups. The results may partly be due to the long term toxic effects of SM on testosterone biosynthesis. Further investigation is strongly recommended.

**Keywords:** Testosterone, Hypogonadism, Glucocorticoid, Mustard gas, Asthma, and Lung diseases

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## Introduction

The hallmark of bronchial asthma disease is chronic airway inflammation, which is characterized by bronchial hyperresponsiveness.<sup>1</sup> Sulfur mustard (SM) is a dangerous chemical warfare agent that was used in the Iraq-Iran conflict, 1980-1988. The respiratory system is one of the most common target or-

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gans affected by the toxic effects of SM. Previous studies have shown that asthma develops among veterans exposed to SM gas.<sup>2-4</sup> Recent epidemiological evidence suggests that sex hormones influence the modulation of immunologic inflammation,<sup>1</sup> the severity, sex, and age related incidence of asthma.<sup>5,6</sup> Hypogonadism has been reported as a short term toxic effect of SM,<sup>7,8</sup> limited chronic inflammatory diseases and a few pulmonary disorders, and also the concurrent use of certain drugs.<sup>9-12</sup> Administration of glucocorticoids is an essential strategy in asthma management;<sup>13</sup> evidence from recent reports indicates that it lowers testosterone biosynthesis.<sup>14,15</sup> This paper however is a new insight into the long term effects of SM on the status of free testosterone levels among SM induced chronic persistent asthma war veterans under glucocorticoid treatment. No similar study has yet been carried out.

The purpose of this survey was to evaluate the status of free testosterone (FT) among SM induced chronic persistent asthma patients with respect to two control populations, the non-exposed asthma group, and the normal age and sex- matched subjects following a relatively long period of primary exposure.

### Material and Methods

This was a cross-sectional, case-control study conducted in 2004 in the Loqman Hakeem Teaching Hospital of the Shaheed Beheshti University of Medical Sciences, Tehran. The target population were war veterans exposed to SM between 1983-1988, and, as a result had developed asthmatic diseases. Selection of participants was carried out from among the chemical victims referred to outpatient clinics; self-reported questionnaires were used.

The questionnaires contained demographic data, history of SM gas exposures such as symptoms of chemical contact, conflict zones, type of chemical gas, hospitalization, wartime contact, and the occasions of chemical episode, complications of respiratory sys-

tem following exposure(s), standard questions concerning asthma symptoms,<sup>16</sup> doctor diagnosed respiratory diseases, existing asthma in past 12 months, usage of the glucocorticoids as oral and or inhalation routes, dosage of drugs used, and period of recommended therapy, history of reproductive system neoplasia, history of usage of sex-hormones (testosterone), liver disease, endocrinopathy disorders (hyperthyroidism, diabetes mellitus), and kidney disorders. The questionnaires were completed by subjects in order to demonstrate SM induced asthma population, with other types of disease being omitted. Participation rate was 32%; one physician documented SM exposure (via face-to face interview) and general physical examinations, while another a pulmonologist diagnosed asthma disease. All participations were followed using standard chest x-rays, pulmonary function tests, and, if necessary, reviews of previous medical records. Asthma diagnosis was based on guidelines of the American Thoracic Society (ATS) criteria.<sup>17</sup>

The exclusion criteria included female gender, any history of castration, trauma to testis, history of testis varicosis, usage of sex hormones and/or chemotherapy, existing neoplasia of prostate cancer, diabetes mellitus, hyperthyroidism, liver diseases, nephritic syndrome, history of asthma less than three years, of any acute illness one month prior to study. Glucocorticoid usage should be equal or less 10 mg per day and no longer than one year. All participants in the case and control groups with asthma received different kind of glucocorticoids which adjusted the dose.

Forty-three subjects fulfilled the criteria and were enrolled in the study. The first control group consisted of forty-six subjects with non-exposed asthma, selected from outpatient respiratory clinics; they were age-and sex-matched with the case group. Criteria of the study were adjusted exactly.

The second control group of forty-six, consisted of healthy subjects, age- and sex-matched with the cases. Criteria of the study

applied to volunteers. All subjects gave informed written consent.

Venous blood samples were collected after overnight fasting between 8 A.M to 10 A.M and all blood samples were assessed at one laboratory. FT serum concentrations were assessed with using the Enzyme Linked Immunosorbent Assay (ELISA) method with the use of IBL set (Immuno Biological Laboratorie kit). Levels of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), were assessed using the Radmin kit (made in Italy). Cut off point values for FT were 3.8-34 pg/mL, for FSH 1.3-11.8 mIU/L, and LH for were 1.5-10mIU/L, respectively.

Data collected were documented were presented as means  $\pm$  SD and analyzed using the SPSS version 13. Comparisons of means between groups were performed by ANOVA and Post hoc (Tukey HSD) tests.  $P < 0.05$  was considered significant.

## Results

In the SM induced chronic persistent asthma group, which included 43 male participants, mean age was  $53.95 \pm 6.80$  years and age range was 42-66 years, mod 50-59 years. The mean FT, FSH, and LH, levels were  $15.7 \pm 10.54$  pg/mL,  $11.91 \pm 9.21$  mIU/L,  $10.33 \pm 7.46$  mIU/L, respectively. The frequency of lower than normal level of FT in serum was found in 14, (32.6%) subjects.

Dermography data included the areas of Halabcheh 30.2% (13), the Sardasht 27.9% (12), the Majnon Islands 25.6% (11), and other areas (7) 16.3%. The sequels of SM exposure consist of skin dermatopathy (25) 58.1%, and ocular injury (33) 76.7%. Children of participants were  $2.3 \pm 1.18$ . Mean duration of asthma was  $6.40 \pm 1.92$  years. Peak flow rate was  $312.09 \pm 65.44$  L/s. The type of GC medications prescribed included fluticasone inhalers (26) 60.5%, prednisolone tablets (20) 46.5%, and and beclomethazone inhalers (20) 46.5%.

Forty-six healthy, male subjects were enrolled as the control group, mean age  $53.22 \pm 6.92$  years, age range 42-66 years. The means for FT, FSH, LH, levels were  $22.73 \pm 8.30$  pg/mL,  $6.46 \pm 1.29$  mIU/L,  $5.45 \pm 0.99$  mIU/L, and, respectively.

The non-exposed asthma group consisted of 46 males, without SM exposure. Mean age was  $52.78 \pm 6.30$ , age range 42-66 years. The levels of FT, FSH, LH, and in serum observed were  $16.97 \pm 10.15$  pg/mL,  $10.65 \pm 9.88$  mIU/L and  $7.69 \pm 7.20$  mIU/L, respectively. Eleven subjects (23.9%) had FT below normal. Duration of asthma involvement was  $5.72 \pm 1.61$  years. The PFR was  $302.39 \pm 63.25$  L/s. Children number was  $2.74 \pm 1.08$ . The type of GC medications used included prednisolone tablets (20) 43.5%, beclomethasone inhalers (25) 54.3%, and fluticasone inhalers (20) 54.3%.

The one way ANOVA test was performed to compare means of FT, FSH and LH levels of the three groups; significant differences were seen between FT, FSH, and LH levels of groups,  $P < 0.002$ ,  $P < 0.003$ , and  $P < 0.001$ , respectively. Post hoc test (Tukey HSD) was carried out between the study groups and significant differences were found between the FT levels of the SM induced asthma and the normal healthy groups ( $P < 0.002$ ); this was also the case for differences found between non-exposed asthma and normal groups ( $P < 0.014$ ). Tukey HSD test was also performed to compare FSH, and LH levels. Significant differences were seen between FSH levels among the cases and normal subjects ( $P < 0.004$ ), and between the non-exposed asthma and the normal groups ( $P < 0.03$ ). There were significant differences between LH levels of the case the normal groups ( $P < 0.001$ ) (Table 1).

**Table 1. ANOVA results of comparison between study groups**

Groups		Number	Mean $\pm$ SD	P value
FT Pg/mL	Case	43	15.7 $\pm$ 10.54	0.002
	Non-exposed asthma control	46	16.97 $\pm$ 10.15	
	Healthy control	46	22.73 $\pm$ 8.30	
LH mIU/L	Case	43	10.33 $\pm$ 7.46	0.001
	Non-exposed asthma control	46	7.69 $\pm$ 7.20	
	Healthy control	46	5.45 $\pm$ 0.99	
FSH mIU/L	Case	43	11.91 $\pm$ 9.21	0.003
	Non-exposed asthma control	46	10.65 $\pm$ 9.88	
	Healthy control	46	6.46 $\pm$ 1.29	

FT= Free Testosterone, LH= Luteinizing Hormone, FSH= Follicle Stimulating Hormone.

## Discussion

A significantly higher frequency of serum FT below normal indicating hypogonad hypogonadism, was found among SM induced asthma (32.6%), compared to the non-exposed asthma control group (23.9%). The results may be consequences or effects of several background factors that maybe found among SM induced asthma veterans population.

Sulfur mustard gas is known as a highly dangerous chemically warfare agent, that was used in the Iraq-Iran war between 1981 and 1988. Data from various studies has shown that the toxic effects of SM affects spermatogenesis in short term;<sup>7,8</sup> abnormal spermatogenesis among SM populations hence, is one of the known risk factors in the development of hypogonadism. Low FT in the serum of SM induced asthma patients may partly be related to the longterm effects, probably life-long toxicity. We were unable to evaluate the spermatogenesis status of our population.

Our results are in agreement those of other studies on GC effects. Previous data support reports that GC therapy can lower testosterone biosynthesis<sup>14,15</sup> and influence the pituitary response to gonadotropin releasing hormone (GnRH).<sup>18</sup> Gluco-corticoids is prescribed for the management of chronic persistent asthma. Despite, the duration of

asthma and usage of GC being very similar in two recent groups, the significant differences results was probably not related to direct GC effects alone.

The study was performed 15 years following SM exposure. Variations due to age may be another factor affecting the results observed. Data from two of the largest longitudinal studies regarding the effects of aging on T levels show that it leads to declines in both total and bioavailability circulating T levels at a relatively constant rate, importantly, independent of obesity, chronic illness, prescription medications, prostate problems, cigarette smoking, or alcoholism; rate of decline in T levels was reported to be 1-2% annually.<sup>19,20</sup> The age range of our population was between 42 to 66 years; hence considering the period of asthma involvement in both groups and the duration of expoure, changes caused by age apparently had relatively limited effect on decreasing T levels.

Recent studies demonstrate that circulating testosterone levels are suppressed by physical and psychological stress.<sup>20,21,24</sup> Post-traumatic stress disorder (PTSD) is a psychiatric disorder that was a significant finding among veterans, especially following toxic exposure.<sup>22,23</sup> Recent reports however show there was no decrease in testosterone levels in chronic combat related-PTSD, probably a result of the mechanism of the adaptation of the

Hypothalamus-pituitary axis in chronic conditions.<sup>21</sup>

In conclusion serum FT was significantly below the normal range in the exposed group as compared to the non-exposed asthmatic groups. The results suggest FT needs regular monitoring, particularly in SM exposed vet-

erans, especially during their reproductive years.

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