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TRANSIENT DECREASED SERUM TESTOSTERONE AFTER EXPOSURE TO MUSTARD GAS*

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ABSTRACT

The effects of exposure to chemical weapons containing sulfur mustards on the serum levels of total and free testosterone, gonadotropins and prolactin was investigated in young Iranian men. In the first five weeks after injury both serum total and free testosterone were significantly decreased as compared to control values. Three of 13 men had total testosterone below 300 ng/dL; and two of six had subnormal serum free testosterone concentrations. By the fifth week after exposure serum free testosterone did not change; however there was further fall in mean serum total testosterone and 70% of men had subnormal values. Both serum total and free testosterone concentrations returned to normal values by the 12th week after injury.

There was a significant rise in serum FSH and prolactin by the fifth week and in serum LH by the fourth week after exposure. These data suggest that injury by the chemical warfare containing sulfur mustard may cause acute inhibition of testosterone secretion from the testis leading to a significant decline in serum total and free testosterone and some increase in gonadotropin concentrations. The inhibition seems to be transient and hormone levels return to normal by 12th week after injury.

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INTRODUCTION

Undesirable side effects of chemical weapons on the eyes, skin, gastrointestinal, respiratory and hematological systems and increased occurrence of neoplasia were reported after the employment of chemical weapons in the First World War.[1,2] Although the effect of nitrogen mustard on the testis has been studied in both animals and humans,[3,5] the effect of exposure to sulfur mustard as a chemical weapon has not been observed. We have previously reported a significant decrease in serum testosterone in men injured by chemical weapons containing sulfur mustard during the Iran-Iraq conflict.[6,7] In order to determine the time course of serum testosterone changes in this condition, we undertook a study of serial measurements of serum testosterone, gonadotropins and prolactin in men exposed to chemical weapons containing mustards.

MATERIAL AND METHODS

All studies were performed in young Iranian men, aged between 21 to 34 years, who were brought to major teaching hospitals of Shaheed Beheshti University of Medical Sciences between two to 10 days after they were injured by chemical weapons. The reported characteristics of the chemical weapon in the field, positive findings using special kits, delayed

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Table I. Serum concentrations of testosterone, LH,FSH and prolactin in 13 young men following injury by the chemical weapons containing mustard

serum concent ation	Weeks after injury		
	1 - 2	3 - 4	5
Testosterone (ng/dl)	404 ± 152	406 ± 136	225 ± 151*
LH (mU/ml)	7.2 ± 4.2	14.6 ± 4.8	4.9 ± 3.7
FSH (mU/ml)	5.7 ± 3.6	11.7 ± 5.9*	16.4 ± 8.7*
Prolactin (ng/ml)	7.3 ± 4.4	9.7 ± 10.3	14.3 ± 5.7+

* $P<0.001$, + $p<0.005$, compaired to values on the 1-2 weeks after injury, using paired t test.

appearance of symptoms (several hours) and characteristic toxic effects on the skin, eyes, and respiratory system confirmed that the chemical weapons employed contained mustards. The patients did not receive opiates and steroids during observation.

In the first study, serum concentration of testosterone, FSH,LH and prolactin were measured in 13 men injured by chemical warfare. First blood samples were drawn upon arrival in the first two weeks after injury. Second blood tests were done two weeks later and the third on the fifth week following exposure.

In the second study, serum concentrations of total and free testosterone were determined in six injured men up to twelve weeks after they had been exposed to chemical warfare containing sulfur mustard.

A group of 18 healthy age-matched men were used as normal controls. All hormone determinations were determined by commercial kits. For each day three samples were taken every twenty minutes and the mean of the three values was considered as the hormone concentration. The sera obtained on various dates were kept frozen and were processed on the same day.

For statistical analysis, t test was performed to compare values between injured and normal controls. A paired t test was used to compare values in one subject on different dates. This study had been approved by the Ethical Committee of the University.

RESULTS

In the first study, serum concentration of testosterone was decreased in the first two weeks after exposure to mustard, as compared to control (404 ± 152 vs 773 ± 245 ng/dL respectively, $p<0.001$). Three of 13 men (23%) had serum testosterone below 300 ng/dL. There was no significant change in the third and fourth week samples (Table I); however by the fifth week following injury serum testosterone had further decreased to 225±151 ng/dL ($p<0.001$, as compared to the values of the first and second weeks after exposure), and nine of 13 men (70%) had testosterone levels below 300ng/dL.

Serum concetrations of FSH, LH and prolactin in the first and second week samples (5.7±3.6 mU/mL, 7.2±4.2 mU/mL and 7.3±4.4 ng/mL respectively) were not statistically different from those of normal controls (10.1±3.2, 8.3±2.1 and 13.9±5.5 respectively). There was a significant increase in serum prolactin from 7.3±4.4 in the first two weeks to 14.3±5.7 ng/mL in the 5th week after exposure ($p<0.005$). Significant rise in serum FSH from 5.7±3.6 in the first and second week period to 16.4±8.7 mU/mL in the fifth week ($p<0.001$) was also observed. Mean serum concentration of LH increased from 7.2±4.2 in the 1-2 week to 14.6±4.8 mU/mL in the 4th week ($p<0.001$). However by the 5th week it had decreased to 4.9±3.7 mU/mL, not significant from values in the first and second week period after exposure.

In the second study the fall in serum total testosterone was again observed (Table II). Serum testosterone concentration decreased from 454±176 in the first two weeks to 217±183 ng/dL in the fifth week after exposure ($p<0.001$). By the fifth week after exposure, four of six men had serum testosterone below 150 ng/dL.

Table II. Serum total and free testosterone concentrations in six young men, measured up to twelve weeks following exposure to chemical weapons containing mustard

Patients (no)	Serum testosterone concentration (ng/dl) weeks after exposure					Serum free testosterone conc. (pg/ml) weeks after exposure				
	1-2	3-4	5	7	12	1-2	3-4	5	7	12
1	400	453	328	304	557	29	35	35	38	40
2	441	584	141	290	447	20	23	23	24	33
3	355	321	140	340	823	19	29	12	21	46
4	217	187	46	632	608	18	18	21	20	26
5	701	419	540	592	652	33	40	40	41	43
6	613	589	111	463	588	31	38	40	42	48
Mean	454	425	217	451	612	25.0	30.5	28.5	31	39.3
SD	176	155	183	137	124	6.7	8.7	11.5	10.4	8.4

Mean ± SD in 18 normal age-matched men were: total testosterone 773±254 ng/dl; and free testosterone 35.5 ± 11.1 pg/ml.

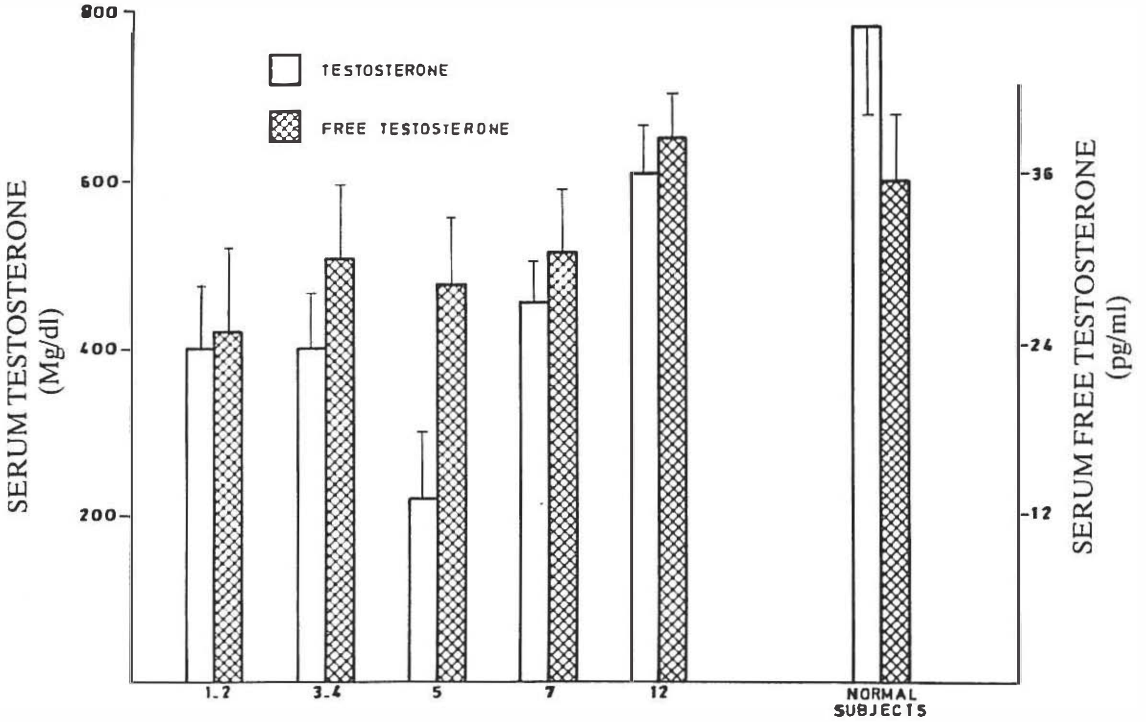

Fig.1: Effect of exposure to chemical weapons containing sulfur mustards on mean (± SEM) serum levels of total and free testosterone in 6 men. The data are subdivided according to post-injury interval. Serum total testosterone is markedly decreased from 1-2 through 7th week p(<0.001); and serum free testosterone is significantly decreased 1-2 weeks after exposure ($p<0.05$), as compared to normal controls. Both total and free testosterone values return to normal 12 weeks after injury.

However by the seventh week serum testosterone increased to 451±137 ng/dL and only one man had low serum testosterone. Serum testosterone concentration rose to 612±124 ng/dL by the 12th week and all subjects had serum testosterone in mid-normal range. Mean serum free testosterone concentration was significantly decreased in the first and second week period after injury as compared to that of normal controls (25.0±6.7 vs 35.5±11.1 pg/mL, $p<0.05$). The range of serum free testosterone concentration was between 19.9 to 60.5 pg/mL in 18 healthy men. As shown in Table II free testosterone was below normal range in two and borderline low in one patient, 1-2 weeks after exposure to sulfur mustard. There was no significant change in serum free testosterone up to five weeks after injury. Thereafter, mean serum free testosterone concentration increased to 31±10.4 and 39.3±8.4 pg/mL by the 7th and 12th week respectively. Mean free testosterone concentration in the 12 week after exposure was significantly increased as compared to that of 1-2 weeks (39.3±8.4 vs 25.0±6.7 pg/mL, $p<0.001$), but it was not significantly different from that of normal control (Fig. 1).

DISCUSSION

To determine the effect of chemical warfare containing mustards on serum levels of gonadotropin, prolactin and total and free testosterone, we measured serum concentrations of these hormones in young men injured by vesicants. The major finding in this study has been a significant fall in both total and free testosterone in the first few days after exposure to mustard which persisted for five weeks and returned to normal by 12 weeks after injury. Mean serum total testosterone one to two weeks after injury was 52 and 55 percent of mean normal values and decreased to less than 30% by the fifth week after exposure to mustard. Mean serum free testosterone was also decreased to 72% of mean normal values a few days after injury but no further decline was observed up to the fifth week following exposure. The discrepancy between the fall in total testos and no change in serum free testosterone in the subsequent weeks, may point to the fact that further decrease in serum testosterone from the first to fifth week after injury is due to decreased sex hormone binding globulin (SHBG).

A straightforward explanation for the nature of decline in serum testosterone levels after exposure to chemical weapons containing sulfur mustard can not be proposed. However the fall in both total and free testosterone a few days after injury points to a decrease in testicular secretion of testosterone. Normal serum concentrations of gonadotropins upon admission and slight but significant rise in serum LH three to four weeks and in serum FSH five weeks after exposure to vesicants suggests a primary testicular failure. However we can not explain the subsequent fall in serum LH from the third and fourth weeks to the fifth week after injury while both serum total and free testosterone were still low five weeks after exposure to mustard. The rise in serum prolactin on the fifth week after injury could be attributed to the stress of disease involving multiple systems.

During World War I it was learned that the sulfur and nitrogen mustards have toxic effects on various tissues in man.[8] Studies on the effect of nitrogen mustard on various organs including the testis began when this agent was used for the chemotherapy of Hodgkin's disease and other lymphoproliferative disorders. Testicular atrophy and impaired spermatogenesis were observed in the postmortem examination of patients receiving nitrogen mustard as the only chemical agent and in experimental animals with varying doses of the various nitrogen mustard preparations.[3,4,9] The effect of combination chemotherapy including nitrogen mustard on fertility and gonadal function has also been reported.[5,10] However, in all human studies nitrogen mustard and other alkylating agents were employed for many months as cyclic treatment for lymphoproliferative disorders. In the case of exposure to the chemical warfare the exposure to vesicant occurs within a few seconds or minutes and is never repeated. No data is available regarding the effect of nitrogen mustard alone on serum testosterone concentrations. In one combination chemotherapy using nitrogen mustard along with vinblastin, procarbazine and prednisolone the mean serum total testosterone was reported to be within normal limits following cessation of chemotherapy.[10]

Serum concentration of testosterone may also fall after head injury, multiple trauma, surgical stress, opiate and steroid administration.[11,12] Our previous studies showed that subjects exposed to chemical warfare containing nerve agents showed no alteration in serum levels of testosterone, prolactin and gonadotropins.[6] Therefore, the present findings could not be attributed to the stress of war and injury.

The decline in serum testosterone observed in the present study is somewhat similar to that reported after burn trauma.[13,16] Serum total testosterone levels are very low in the first few weeks after burns, and return to normal values during convalescence three to 18 months later.[11,14]

The toxic effect of sulfur mustard was originally attributed to its hydrolysis and the liberation of free hydrochloric acid or to the formation of the highly reactive cyclic onium cation.[17] Sulfur mustard also depresses and inhibits many of the vital enzymes in various cells. However, it seems likely that the characteristic cytotoxic effect of alkylating agents, including mustard, is due to their ability to crosslink the twin strands of the DNA replication.[18] It is conceivable that disturbances in DNA replication result in testicular damage and transient malfunction in the Leydig cells resulting in decreased secretion of testosterone following exposure to chemical warfare containing sulfur mustard.

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